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Cactus bushes in Tunisia
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Ornithomimus, a coelurosaur from the Upper Cretaceous Did dinosaurs run, walk of lumber? See page 129. [Drawing by Tony Thulborn.]

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Rattlesnake blood plasma could be used against snakebite.
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A new skull, of *Homo erectus*, has been discovered in East Africa and is here contrasted with *Australopithecus* (background). See pages 541 and 572–576.

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Sheep grazing can cause an increase in kangaroos, and the kangaroo's future depends on the little appreciated fact that they can be conserved on grazing lands without posing a threat.

(CSIRO Photo.)

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Class structure in science

SHOULD universities be more responsive to the needs of industry, or is it their job to pursue learning wherever it takes them and to respond to student needs whatever they be? These questions have been asked with increasing insistence in recent months, although probably as a substitute for the more fundamental question: why does industry not appeal more to university graduates, particularly science graduates?

This is hardly simply a British problem; there has been violence in the streets in France recently over moves to direct students into courses with a higher technology content to satisfy French industrial needs. It does, however, manifest itself acutely in Britain. Class distinction and class warfare are an ubiquitous constituent of the British scene, leading to rigid boundaries and ignorant contempt. It is difficult to escape the conclusion that science has its class system too, with universities looking down on the scientific civil service, which in its turn looks down on industry. And mobility, surely the main way of breaking down a class structure, is declining all the time as jobs in universities and the civil service, with their prized security of tenure and excellent pension schemes, become increasingly scarce. Coupled with this, industries based on the physical sciences seem inexorably to be moving away from the research side of their commitment to research and development in the belief that there is not much more in basic science, as we understand it at present, which is convertible into industrial benefit. (This is a view which the Science Research Council (SRC) has also floated recently.) So the outlook is, if anything, for even more broadsides from the ramparts in the future.

It no doubt riles universities to see what they regard as a slackening regard for the pursuit of knowledge in the industrial sector. It no doubt riles industry to see universities continue to regard their best graduates as most suited to academic research. And it no doubt riles the civil service to find itself in the cross-fire and, at present, rather unloved. But the need is for a certain amount of give and take on all sides if the distinctions are not to become more rigid and more bitterly felt.

Manpower planning has a role to play in the rapprochement. Part of the argument in France has been over the payment of differential grants to favour students prepared to take technological courses. A very discrete step in this direction was made by the SRC recently in proposing that at some time in the future it might pay certain postgraduates more than the standard grant. This has brought a predictable response from some student egalitarians, but is a sensible way of trying to raise the quality of graduates in fields where external salaries make the student who sticks to an academic course feel distinctly unequal. Now Mr Gerry Fowler, a minister at the Department of Education and Science, may have stepped back even from such tentative efforts by declaring that the matching of higher education to the manpower needs of the economy is not practicable. Indeed it is not, in a detailed way (and Mr Fowler's arguments seem aimed at detail and precision); but the case for manpower forecasting is not one which admits of too much detail, and meanwhile the half-filled science laboratories, which were built only ten years ago at government urging, are to stay half full.

These low numbers mean that the quality of university science graduates who present themselves for employment in industry is bound to decline, and industry will start to produce its own prescriptions for setting universities to rights. Just as the British workman would love to see the aristocrat reduced to productive manual labour, so industry will, with increasing fervour, call for the university to become more relevant to industrial needs. There have already been salvos from industry about postgraduate education and some of these have at least produced some sensible results. But the sort of ideas floated by Dr Marion McQuillan, of Imperial Metal Industries, in a lecture to the Research and Development Society in London last week, need to be resisted firmly. Dr McQuillan proposes that funding for university research projects should only be approved if the research has a clear economic benefit. Phrases on grant applications stating that the purpose of the work was "a better understanding of . . ." should not be acceptable. Individual universities should be designated as centres of expertise for certain industrial research purposes.

Maybe the idea was presented tongue-in-cheek. Certainly there are university departments that need a vigorous shake, but to seek to distort the function of a university so much (it would be rather like a vice-chancellor urging industry to give up profit making) serves no useful purpose beyond convincing people in universities that there is ignorance outside the walls. Up comes the drawbridge and class warfare takes another step forward.

Dishonesty and grants

Leigh Van Valen, Professor of Biology at the University of Chicago, is Editor of the journal Evolutionary Theory *and is well known for his criticisms of "irresponsible" scientific publishing. Here he comments on an aspect of the American grant system which is also damaging to scientific research*

VARIOUS defects of the system of research grants, as it is practised in the United States, have been discussed extensively. I wish to focus on one which is rarely mentioned, although widely known, and which may well be the most serious. This is the incompatibility, in practice, between honest grant applications and conceptually original work.

The incompatibility is as follows. Any conceptually innovative scientist with some experience knows that there is a good probability that in the next year or two he will have one or more new ideas which will change the direction of his research. In practice, the National Science Foundation and the National Institutes of Health do not tolerate much deviation from the plan of research in the original proposal. The scientist must therefore either adhere to the original proposal at a loss to science, or do his best science while deviating from the conditions under which his grant was awarded.

Two alternatives

Most scientists resolve the situation in one of two ways at the time of applying for grants:

- Original workers who do participate in the granting process use various conscious or unconscious deceits. One kind is a proposal to do research which has just been done or will be done before the grant arrives. This is very common (M. L. Wolbarsht, *Science*, **185**, 399; 1974). Another common kind is a proposal to do research which will be done only if no new ideas come before the grant ends. For both these ploys, and others, it is empirically necessary that the nature of the ploy not be stated This is what creates the dishonesty. When the granting agency explicitly knows of such reservations, or if the application is general enough to be honest, the application is automatically rejected. This is of course the same mentality that required J. D. Watson to falsify his NSF-administered postdoctoral fellowship, where he did part of his Nobel Prize work that he couldn't have done if he had acted honestly.
- Research which really is predictable, at least in outline, is common and is the backbone of most scientists most of the time. There is no problem in such cases. One really can know that one will probably be able to work out the evolution of a group, or test an established hypothesis, or determine whether a known phenomenon applies in a particular case. Because the work is predictable, even though the results may not be, it is usually less than intellectually exciting. It may nevertheless have important results, and it really is necessary for the progress of science. Being easily accountable, it gets funded. Exciting work by honest people does not get funded. This may perhaps take its place among the laws of nature, at least within the boundaries of the United States.

Why should the subject of a grant be adhered to when science is better served by deviation? The administrative answer is accountability: a grant is given for a specific purpose. In applied science this is certainly appropriate. Here the purpose of the money is determined by governmental priorities, by the government's view of national (and occasionally even human) needs, In pure science, however, the aim of giving the money is the advance of scientific knowledge. It is this very advance which is hindered by the present practice. A diversity of approaches is valuable but is not permitted.

The heart of science is new ideas, and their development is discriminated against. This is not true in Europe, whatever the defects of the systems there. My European visitors, who have come from every major country, are uniformly astonished that the United States Government refuses to support creative science. The problem is as real for Szent-Györgyi (*Science*, **176**, 966; 1972), with a Nobel Prize to back him up, as it is for others. I have had several useful ideas in biology and mathematics. None were developed under a government grant, and none could have been. I have also had several grants and eventually realised that the required conformity stultified my research.

Three categories

What to do? I suggest that there be three categories of grants:

- Most grants would be based on specific proposals, as now, but with the explicit understanding that new ideas may be followed at the scientist's discretion. Specific restrictions on such freedom may be necessary in rare cases.
- Specific proposals are inappropriate for much theoretical work; when one knows just what one will do, it is done. This is quite literally true. In such cases grants could be awarded on the basis of research done in the past three years or so, with allowance for extenuating circumstances. The second category would also be appropriate for some scientists who are beginning to work in an area different from their previous work.
- For beginning theoreticians, a modest amount of money for computer costs and the like could be provided for a year or two. These three proposals aim to minimise waste of money while endeavouring to maximise the progress of science.

Many scientists are perfectly comfortable with the present situation. Some of them are the Appollonians of Szent-Györgyi, who "develop established lines to perfection". Others are Dionysians, who know "only the direction in which [they want] to go into the unknown", but who have reconciled their dishonesty with survival. For it is often a matter of survival. Rationalisation of dishonesty is therefore easy.

We recently removed a President because he was dishonest. The norm in our science remains dishonesty, because it is made necessary for the survival of creative research. Often one may be honest or continue in science, but not both. The choice applies mainly to the more creative scientists. We can easily resolve the dilemma if we so want. Do we? □

The Soviet energy balance

Philip Hanson surveys Soviet energy resources and assesses the USSR's energy policy as a factor crucially affecting the prospects for economic growth

OF ALL the countries in the world, the Soviet Union has the most impressive known endowment of non-agricultural natural resources. This is as true of fuel as it is of raw materials. In addition to being the world's second largest gold producer and a leading producer of almost all the useful mineral raw materials, the USSR has all the energy options available from domestic sources: wood, peat, oil shale, coal, oil, natural gas, hydro power and uranium.

In the past few years, however, when energy supplies have become a crucial consideration in economic policy everywhere, the Soviet energy balance has begun to seem precarious. Nobody disputes that the Soviet Union still has vast "potential", or geologically indicated, reserves of the major fuels. Beyond that, however, the Soviet energy position is open to a variety of assessments.

One problem is reserves. The extent of proved reserves, on definitions comparable to those used in the West, is not clearly established. Another problem is costs. The main areas from which increases in fuel supply can come are east of the Urals. Extraction costs in the new oil, gas and coal fields of Siberia vary greatly: for some deposits costs are quite low, for others rather high. But the social overhead costs of bringing roads, housing and amenities to these areas are generally high. Once the fields are being exploited, the cost of moving Siberian energy to the user (by oil and gas pipelines, rail shipments of coal and high-voltage electricity transmission) are generally very high. The alternative of moving the users to the energy supplies is also costly. These high costs are attributable mainly to the harsh natural conditions of most of the area—notably blizzards, permafrost and swamps.

Costs and reserves are not the only contentious issues. There is a good deal of uncertainty about the demand side. How fast will Soviet energy consumption rise in the next five or ten years? Will the Soviet Union remain a net exporter of energy in general and of oil in particular? What are the chances of the West obtaining significant energy supplies from the USSR? The wide spread and frequent revisions of recent forecasts of Soviet energy consumption in 1980 do not inspire much confidence in such forecasts. And the implications for Soviet energy supplies to the West also depend on future Soviet policies in the supply of energy to Eastern Europe, which are not easy to predict beyond 1980.

There is another problem in any assessment of Soviet energy prospects, and this concerns technology. How badly do the Russians need Western technology in order to increase their oil, gas and coal production? Do they need it enough to ensure that some of the more grandiose East-West co-operation projects in Siberia will actually materialise?

Those are the main questions. Some can be answered with reasonable confidence; others are wide open. The accompanying tables summarise recent and planned developments in Soviet energy supply in the 1970s. The plans for 1976–1980 envisage some slowdown in the growth of the primary fuels, except for coal. With petroleum output in some of the older fields in the European USSR expected to fall, this is not too surprising. Oil and gas production in West Siberia (Tomsk and Tyumen provinces) is the main source of growth in the immediate future. West Siberia produced 148 million tonnes of oil and 38,000 million cubic metres of natural gas in 1975 and these figures are planned to reach 300–310 million and 115,000–145,000 million respectively in 1980.

The high costs and sheer physical limits to West Siberian development in the next few years, however, seem to have led the planners to accept a slight deceleration in the growth of oil and gas production. One additional consideration may have been that proven reserves were not rising fast enough. Soviet oil reserves figures are a state secret, but several recent statements by V. Shashin, the Minister for the Oil Industry, imply that new reserves proved have recently been less than the rate of extraction. There is not much doubt that the oil is there; the USSR has 37% of the world's oil-bearing land. But exploration and proving costs are high and the gap between potential and proved reserves is large. A recent US estimate is that the USSR has a relatively modest 12.1% of the world's proved oil reserves. Reserves figures for the other fuels are not secret, though up-to-date official figures are not always available. One estimate of Soviet proved, semi-proved and probable gas reserves (Soviet categories $A+B+C_1$) at the end of 1974 is 23.8×10^{12} cubic metres. This represents 92 years' production at 1974 rates —an impressive position. Nonetheless, the difficulties of exploiting these reserves are considerable, and production has fallen behind plan.

Soviet energy policy has therefore shifted recently in favour of coal and nuclear power. Major increments to coal output are planned from the Ekibastuz and Kansk-Achinsk fields whose low-cost coal, produced in large part by open-cast and strip mining, can be effectively used as a fuel for thermal

Table 1 Soviet Energy Production

	1970	Annual average growth rate (%)	**1975**	Annual average growth rate (%)	**1980** plan (mid-point of range)
Oil (10^6 tonnes)	353	6.9	491	5.2	630
Natural gas (10^9 m^3)	198	7.9	289	7.7	418
Coal (10^6 tonnes)	624	2.4	701	2.7	800
Total energy (10^6 tonnes sfe)[1]	1,238	6.0	1,656	5.5	2,158
Electricity (10^6 MWh)	741	7.2	1,038	5.6	1,360

[1]Standard fuel equivalent in metric tonnes of 7,000 kilocalories.
Source: Soviet plan fulfilment reports and draft directives for the 1976–1980 plan, except that total energy production figures are author's own estimates.

Table 2 Contribution of Primary Sources of Energy to Total Energy Production (%)[1]

Energy source	**1970**	**1975**	**1980** plan
Oil	41	42	42
Natural gas	19	21	23
Coal	35	30	26
Peat, oil shale, wood	4	7	10
Hydro	1		
Nuclear	½		

[1]Because of rounding, columns do not all sum to 100.
Source: Author's estimates on the basis of the sources used in Table 1.

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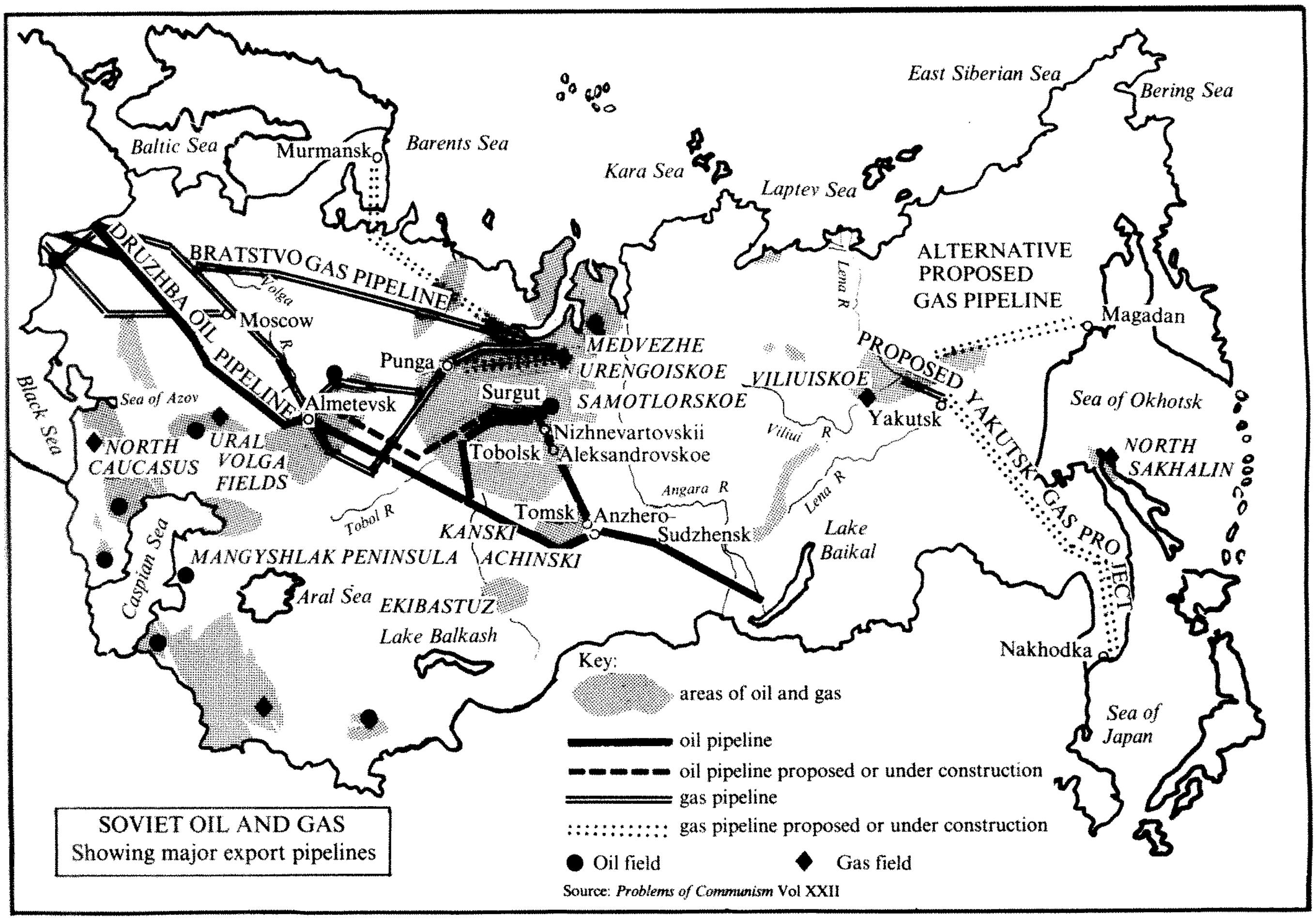


Source: *Problems of Communism* Vol XXII

power stations in the Urals and elsewhere. At the same time the Five Year Plan requires the construction of nuclear power stations in the European USSR and the Urals with total capacity of 13–15 million kW, out of a construction programme of 67–70 million kW of new generating capacity of all types. Soviet installed nuclear power capacity has hitherto been small—3.7 million kW at the end of 1974 compared to 30.4 million in the USA and a total of about 14 million in Western Europe—so this plan would increase the role of nuclear power stations considerably.

This shift in policy has been indicated in a number of Soviet sources over the past two years. It appears to be a response both to higher world energy prices following the Yom Kippur war and to previously unexpected costs and bottlenecks in Siberian oil and gas developments. In his report to the XXV Party Congress in February, Mr Kosygin put the change in quite striking terms: future increases in oil and gas output would be allocated to "technological" needs (for example, feedstocks for the chemical industry), rather than to electricity production. The latter will depend much more heavily on hydro and nuclear power and coal than had previously been planned.

It could well be that the Soviet planners now see energy supply as a bottleneck restricting Soviet economic growth. American recalculations of recent Soviet growth in terms comparable with the measurement of economic growth in the West show an annual growth rate of Soviet real GNP in the first half of this decade of 3.9%. The plan for the latter half of the decade (of a 4.4 to 5.0% annual growth in net material product) probably implies something like a 3½% GNP growth rate. This is admittedly only a modest deceleration, and the plan targets that are finally approved may even show no deceleration at all. But in the light of postwar Soviet plans generally, the 1980 targets look a lot more modest than they do in relation to recent performance; it is likely that they reflect newly-perceived constraints of some severity.

In the early 1970s Soviet electricity and total energy production grew appreciably faster than total national output. In part this reflected the fact that the net export of energy grew in most of the past five-year period. It probably also reflected a tendency for energy consumption to rise somewhat faster than GNP. If Soviet energy consumption continues to rise slightly faster than GNP, the exportable surplus may not grow at all between 1975 and 1980.

A recent study by Jeremy Russell projects a Soviet energy surplus in 1980 of 200–250 million tonnes of conventional fuel equivalent; a surplus of the same order of size on that of 1975. For oil alone the surplus is likely to narrow. Russell's conclusion is that the Comecon group as a whole will still have a surplus of oil production over consumption in 1980 but that a Comecon oil deficit, that is, net imports, is likely fairly soon thereafter.

Soviet long-term oil delivery contracts to Eastern Europe up to 1980 have already been agreed. It is likely that in 1980 only 20–30 million tonnes of Soviet oil (according to Russell), or perhaps 35 million tonnes (according to the Brussels market research organisation, *east-west*), will be available to Western markets. The USSR itself might remain a small net exporter of oil, and some imported Middle East oil might be routed to Eastern Europe through Soviet pipelines.

The position could be quite different as far as natural gas is concerned. Russell projects an annual net Comecon export of natural gas—essentially Soviet natural gas—of perhaps 30–50,000 million cubic metres a year in the early 1980s. Soviet natural gas is already being delivered by pipeline to Austria, Italy, West Germany and France, and Iranian natural gas is being piped through the USSR to West Germany, France and Austria. These are both long term deals and could involve natural gas supplies of the order of 20,000 million cubic metres a year to Western Europe in 1980. The Siberian gas deals that have been discussed with US and Japanese companies in recent years (the Yakutsk and North Star deals) are larger and considerably more problematic. They involve tanker deliveries of liquefied natural gas out of a Pacific coast port and Murmansk, respectively, at a total rate of perhaps 50,000 million cubic metres a year over 25 years. The commercial and political obstacles to the eventual implementation of these deals are formidable. On March 31, however, a Soviet–US–Japanese agreement on joint exploration of the Yakutsk gas deposits was signed, with credits of $25 million each being contributed by the Japanese and US consortia.

The fact that this preliminary agreement has been signed despite all the obstacles indicates the strength of both Soviet and Western interests in the development of Siberian energy supplies. The Western interest requires no explanation, but the Soviet interest is less immediately comprehensible. Why mortgage substantial future energy outputs to the West when energy supplies already form a bottleneck to your own economic growth?

The answer seems to be that Soviet energy production can grow much faster if Western technology is used, and the gains should be enough to repay Western suppliers (in gas) and still have something left over for domestic needs. Soviet technical deficiencies are beginning to show up in a number of key areas. The items which the Russians are unable to produce domestically in sufficient quality and/or quantity in the near future include wide-diameter (1220 and 1420 mm) oil and gas pipeline, pipeline compressor equipment, machinery for deep drilling and secondary oil recovery, submersible pumps, offshore rigs and a wide range of coal mining equipment. It is likely that at least three-quarters of the 20,000 km of wide-diameter oil and gas pipeline laid in 1971–1975 was imported Western pipe. Costly pipe imports continue: in January and February about $500 million worth of new orders were placed in Italy, Japan and France.

The picture of pervasive East-West interpendence which these projects evoke is misleading, however. Soviet natural gas deliveries to West Germany provide less than 15% of West German natural gas consumption. The corresponding figure for the USA in the early 1980s in the event (now remote) of both the North Star and Yakutsk projects going ahead has been put at about 10%.

On the Soviet side, the best assessment seems to be that the Russians will obtain an increase in energy supplies sufficient to support a respectable economic growth rate by a mixture of policies shifting towards fuels less efficient than oil, incurring very large domestic development costs in Siberia, making some use of Western machinery and know-how and perhaps importing significant amounts of Middle Eastern oil. Neither Soviet nor Western energy-supply problems are sufficiently awful to ensure peace on earth and goodwill between Moscow and Washington. □

BRITAIN

Wave energy on

Research in Britain on alternative sources of energy took a step forward last week. Allan Piper reports

THE first real move towards exploiting one of Britain's unconventional energy sources has come with last week's announcement that the Department of Energy (DEN) is to spend £1.01 millions on a 2-year feasibility study of wave-power devices. If the programme is successful further research could lead to the development of such devices to provide up to half of Britain's electrical energy requirements early next century.

News of the venture coincided with the publication of the latest annual report on UK offshore oil and gas resources, which gives estimates that proven reserves of North Sea oil have increased by almost a third over the past year. Although the report revises the production forecast for 1980 slightly downwards, the new estimates are seen as offering the prospect of Britain attaining self-sufficiency in energy by the end of the decade, at the same time providing a stopgap until alternatives such as the wave-power scheme also come on stream.

The decision to press ahead with work on wave-power is based on a National Engineering Laboratory report identifying it as a particularly promising energy resource. Early measurements indicate that the amount of wave energy available around British coasts varies between 40 and 70 kW per metre. The most abundant supplies are offered off north-western Scotland, but areas around the Cornish coast also show considerable potential. One advantage of wave power over other novel energy resources is that the wintertime peak of supply will coincide with maximum consumer demand.

The feasibility study is to assess the relative merits of four possible devices. Three of them, including the "Salter Duck", are British designs; the fourth is Japanese. All use different methods of converting wave energy to a usable form. Each one will be studied at a one-hundredth scale by teams of academic and industrial scientists. While looking principally at operational efficiencies and seaworthiness under varying conditions, they will also estimate approximate costs for any further research and development leading to seagoing trials.

A support programme will cover the collection and analysis of wave data, the long term effects of prolonged wave action on the structures, anchoring and mooring problems, likely environmental and navigational effects of large-scale installations, and possible modes of power generation and transmission. The Central Electricity Generating Board has already started research into ways of integrating the supply into the national grid system.

Commenting on the decision to proceed with wave-power research, Dr Walter Marshall, Chief Scientist at the DEN, said that his department would retain control over the research teams through its Harwell-based Energy Technology Support Unit. The bulk of the £1 million will go towards the principal research programme; it will not be spread equally, but Dr Marshall gave no clues as to whether any of the devices had yet emerged as a clear favourite.

Stressing that the programme represented only an early exploratory phase of wave-power research, Dr Marshall said that further development depends on the findings of the next two years. If all goes well a 10 MW prototype should be in the sea by 1986, with a full-scale model operating by the 1990s, but Dr Marshall declined to forecast a firm date for commercial power production, as past estimates of time-scales on such major projects have generally been "hopelessly optimistic". He did say, however, that commercial installations could be operating during the next century, and DEN figures show that a 600 mile chain of devices around north-western Scotland could eventually provide about half of Britain's present electrical energy requirements even on conservative estimates. Individual devices, each up to about 30 m across, will probably be linked together in 50-km lengths.

International interest in the project has come from the International Energy Agency, which has asked Britain to take the lead in this area of research. The only other country so far seriously involved with wave power is Japan, but Dr Marshall estimated that the total British expenditure—about £1.25 millions including independent research funding—is around four times higher than in Japan. The figure must, however, be seen in perspective: the DEN could spend almost £125 million on nuclear energy this year alone. All the same, at this early stage, Dr Marshall said, any further spending on wave power is unlikely to contract the time-scale of research.

While the wave-power decision represents a significant Government step in a new energy direction, the DEN is also keeping its options open on other alternative sources of energy—solar, tidal, geothermal and wind. It is already engaged on two low-cost areas of research into a possible tidal barrage on the Severn Estuary between Wales and Somerset, and Dr Marshall has expressed a keen interest in solar power.

Even so, it seems clear that the DEN still regards the nuclear, coal, and oil and natural gas options as the mainstays of any future national energy strategy. Dr Marshall believes that the overall cost of wave-power installations could double those of nuclear generators providing comparable power, and his department remains committed to the fast breeder reactor. Additional hopes are pinned on the plan to boost coal production over the next ten years.

As for oil, the new production and reserve estimates are aimed at giving even more room for optimism than previously foreseen. Proven reserves are now placed at 1,350 million tons, compared to last year's, 1,060 million tons, and total reserves could be as high as 3,200 million tons. The production forecast, of 95 million to 115 million tons by 1980, is down slightly on earlier estimates, because of difficulties and delays in commercial development programmes. DEN figures on energy trends, also published last week, meanwhile show that total UK energy consumption fell by 7.5% over the last year. Industrial consumption was down during the last quarter of the year by 17% on the corresponding quarter of 1974. □

A STATEMENT last week from British Nuclear Fuels Ltd (BNFL), which has otherwise captured the public eye in recent months through its nuclear fuel reprocessing activity, disclosed that the UK Ministry of Defence has placed with it a contract for the production of supplies of tritium, the radioactive isotope of hydrogen (half life 12.3 years) which is a key material in the production of H-bombs.

Construction is expected to start in June, following planning permission, of a facility at BNFL's Chapelcross Works, Dumfries, Scotland, where a plant originally built for plutonium production is now operated as a nuclear station supplying power to the national grid. The reactor there will irradiate with neutrons lithium metal which will then be treated in the new plant to separate out the tritium. The plant, which will employ fifty people, should be ready by 1980.

Until now, the Radiochemicals Centre at Amersham has bought from France the tritium Britain uses for civilian purposes, while the Ministry of Defence has obtained its military supplies from the USA. The Ministry now says that a domestic source would be more convenient and would save dollars, but it is not clear whether the civilian imports will continue or whether commercial exploitation of the domestic production for export will be attempted.

BNFL itself has meanwhile faced mixed developments on the reprocessing front. The way ahead for the deal BNFL is sharing with Cogema of France to reprocess Japanese nuclear fuel recently became clearer with the resignation of electricity industry representatives from the Japanese negotiating team which has yet to commit itself following the broadening of the deal to include Cogema.

On the home front, BNFL's Windscale site was recently the location for a gathering of several hundred people protesting against the use of nuclear power and, specifically, the fuel reprocessing carried out there.

● If rabies should enter Britain, the threat would be to wildlife rather than the human population. *Rabies*, a report from the Office of Health Economics, urges surveillance to ensure that pets are not smuggled into the UK or allowed ashore from small boats. The danger results partly from the changing habits of an important vector of rabies, the fox, which as a suburban scavenger now comes into closer contact with domestic animals. For those at risk, meanwhile, the rabies vaccine produced in France and currently on trial in Britain could be both safer and less painful than those previously available.

The vaccine is prepared from the Pitman Moore strain of rabies virus, inactivated with β-propriolactone after being grown in cultures of human diploid cells. The vaccine produces high titres of rabies-neutralising antibodies, and shows promise for immunisation both before and after exposure to the disease. It represents a considerable advance on previous vaccines, produced from viruses grown in animal nervous tissue and duck embryos.

● The fluorocarbon arguments continue, and now the Department of the Environment (DOE) has published the results of a preliminary examination carried out with the assistance of Harwell, the Department of Health, and the Meteorological Office.

Based on studies ranging from the likely effects of fluorocarbons on stratospheric ozone to the health hazards posed by an increased influx of ultraviolet radiation, the findings suggest that the continued use of fluorocarbons at 1973 rates even for another century will ultimately destroy up to 8% of the Earth's stratospheric ozone, resulting in a 16% increase in radiation reaching the Earth's surface. This accords well with the conclusion of a similar World Meteorological Report published earlier this year.

Curbs on aerosols were not recommended, but there was a call for manufacturers and industry to step up the search for serious alternatives to fluorocarbons. The DOE report stresses that with so many uncertainties remaining the need for eventual regulations cannot be discounted, through it is hoped that ongoing research will clear up many of outstanding points within a couple of years.

A Minister in the Environment Department, Denis Howell, said (perhaps arguably) that the predicted increase in radiation levels was about the same as that incurred during a move from northern England to the south coast.

● The fluorocarbon report represents just one aspect of the work of DOE scientists, and along with environmental pollution, this department's research and development teams are also busily engaged with transport planning, building research and work on natural resources. From next August they will find themselves under a new Director General of Research when the present incumbent, Mr D. J. Lyons, steps down after five years in office to make way for Dr Martin Holdgate. Dr Holdgate, currently heading the NERC's Institute of Terrestrial Ecology, has experience leading research teams into South America.

BRITAIN

Drug firm depressant

The Labour Party last week unveiled its discussion document on state participation in Britain's pharmaceuticals industry. At least one argument adduced, concerning research, is likely to be disputed. Chris Sherwell reports

STRONG criticism of the level and direction of private sector pharmaceutical research and development in Britain, which is subsidised by the Department of Health and Social Security (DHSS), is contained in the Labour Party's consultative policy paper "Public Control of the Pharmaceutical Industry". The views form just one plank in the platform from which it argues that, in the public interest, the recently established National Enterprise Board should "as a matter of urgency" acquire "at least one" UK-owned company with a substantial interest in pharmaceuticals and use it as a base for expansion of the public sector within the industry. It also suggests that there should be "research-based planning agreements" between domestic and foreign companies and the national health service to determine, among other things, the amount of research undertaken in Britain.

This amounts to a considerably watered-down version of the original Labour proposals for wholesale nationalisation of the industry. Indeed, the working group compiling the document now describes these proposals as "impractical". Even in their moderated form, though, the latest proposals quickly received a blanket condemnation from Mr Michael Peretz, President of the Association of the British Pharmaceutical Industry.

The research aspects of the argument appear to have a fairly pivotal role: as the 62-page document, which devotes a separate chapter to the subject, says, "the long run future of the pharmaceutical industry in Britain depends on the quality and quantity of its research and development"; the UK-based industry's "greatest asset", it adds, is its group of research scientists. Labour's overall argument for public sector control does not necessarily stand or fall on the case it presents in respect of research and development, of course. But if preliminary reactions expressed last week by researchers themselves are anything to go by, the four identifiable strands of that case do not do much to strengthen the overall argument.

The first, and perhaps most important, strand argues that, while the existing system of profit control encourages research expenditure, it is "impossible to be sure" that all such activity is socially useful. The working group says it is "gravely concerned" that key areas of research can be neglected; there is, it says, "a social case for government funding of research into remedies for conditions which are obscure or occur mainly in developing countries"; it is "open to question whether a wholly profit orientated industry always fully serves a social purpose in long term research".

Only one of the directors of research at industrial and privately-financed research laboratories consulted last week expressed any sympathy with this view—and even he refused to allow himself to be less than 98% against the proposals on this count. Similarly, three professors could see little merit in the second strand, which anticipates the view that a mechanism already exists, through the research councils, for the government to sponsor research in areas where no commercial company would take the risks.

The working group says that even with the implementation of the customer-contractor principle, there has only been a "paper transfer of ongoing work"; there must be "changes of policy over research", not wasteful duplication of administration. The danger, it says, is that "priorities will continue as before". Only one respondent tended to agree with this, and then only because he thought the MRC was "in a mess anyway". But no respondent disputed that the mechanism was indeed there.

The Labour group's objections go further, however. It points out that any long term research effort sponsored by a government department in an area of strong social need would have to be carefully planned; but, it says, this might replicate the very divorce of long term research from industry which is currently a weakness. It admits that closer links could not be better achieved by a public sector company which was commercially orientated. But, and this is the third strand, a long term research effort sponsored by a public sector company would be "more defensible for the government", and might also make it "easier to establish closer structural links" between the DHSS. the research councils and the industry. Research staff might also prefer to work in a publicly-owned where profit-maximisation was not the only force guiding their work, it argues.

These suggestions also fell on stony ground because they seemed so much a matter of opinion. Cooperation already existed, *Nature* was told, and and more bureaucracy was not wanted. As for the final strand of the argument, concerning competition and duplication in research, there was no doubt that both were vital to progress, and in fact even the Labour group acknowledges the advantages without seriously challenging them.

Beyond the idea of closer cooperation between a publicly-owned sector and existing state-sponsored research the Labour group wants wide-ranging planning agreements and a "general system of indirect public accountability and control", the objective being "to change the balance of public and private power within the industry". ☐

USA

Explosions treaty blasted

Colin Norman reports from Washington on the controversy blowing up over the latest US–USSR nuclear agreement

IN JULY 1974, Richard Nixon, then almost engulfed by Watergate, completed one of his last Presidential acts of international statesmanship — the signing of a US–USSR bilateral agreement outlawing the testing of nuclear weapons with yields greater than 150 kilotons. The treaty was greeted by arms control advocates in the USA with utter dismay. They regarded it as a meaningless gesture which would do little to dampen the arms race. Last month, that treaty was extended to cover so-called peaceful nuclear explosions and the reaction has been equally negative.

The Federation of American Scientists (FAS), a liberal organisation whose sponsors include a galaxy of scientific stars, has condemned the joint pact as "worse than nothing". And last week, two former high ranking government officials argued that the treaty is little more than a sham which could seriously impede efforts to prevent the proliferation of nuclear weapsons to countries which do not now possess them. Such powerful opposition could delay, or even scuttle, ratification of the treaty by the Senate.

The 1974 agreement signed by Mr Nixon and Mr Brezhnev was not due to come into effect until March 31 this year, and it covered only weapons testing—the question of peaceful nuclear explosions was left aside for further negotiations. The treaty was soon heavily attacked on the grounds that the 150 kiloton limit is too high, that the two-year delay in implementing the pact would allow both sides to get in as much large-scale testing as they wanted, and that the exclusion of so-called peaceful explosions left a loophole large enough for either military establishment to shoot massive warheads through. Most critics urged the Administration to go back to the negotiating table and come up with an entirely new treaty.

That hasn't happened. Instead, the Administration has negotiated an agreement on peaceful nuclear explosions which essentially sets an upper limit of 150 kilotons for them as well, and which leaves the rest of the 1974 pact unchanged. Though the full text of the new treaty has not yet been released, Administration officials have said that it contains a provision allowing on-site inspection by foreign nationals for peaceful explosions which consist of several blasts whose combined yield is greater than 150 kilotons. Advance warning of such events would also have to be given.

Critics charged last week that the inclusion of the provisions covering so-called peaceful explosions in an already unacceptable treaty makes a bad situation even worse. Aside from the statement from the FAS, criticism came from Dr Herbert Scoville, former chief of research and development in the Central Intelligence Agency, and Adrian Fisher, Dean of Georgetown University Law School and former Deputy Director of the Arms Control and Disarmament Agency (ACDA). Fisher was the chief US negotiator for the historic 1963 Partial Test Ban Treaty which ended atmospheric testing by all nations except France and China. Scoville and Fisher fired off their criticisms during a seminar held by the Arms Control Association.

A central complaint is that the 150 kiloton threshold is so high that it poses virtually no constraint on weapons development. Both the USSR and the USA have crammed many high-yield tests into the two-year period before the treaty was due to take effect. This has enabled the United States to develop a new warhead for the Minuteman missile, and both sides now have such a vast range of tested warheads on the shelf that there's little need to conduct further high yield tests. Moreover, weapons development in the next decade or so is likely to concentrate mostly on low-yield tactical nuclear devices, testing of which would be allowed under the proposed treaty. As an arms control measure, the treaty is therefore viewed as ineffective.

In addition, both Scoville and Fisher suggested that the high limit could impede future negotiations in other areas of arms control. The usual US posture in such negotiations is to argue that limits should be determined by the ability of verification measures to ensure that no treaty violations take place. In this case, however, it is generally agreed that the United States is capable of detecting and identifying explosions in the USSR down to about 15 kilotons. (The actual detection limit is a matter of some dispute, but nobody is arguing that the limit is as high as 150 kilotons.) Thus, Scoville argued last week that "we have lost the principle of verification from our stance on arms control".

The USA and the USSR have twice agreed to seek a treaty banning all nuclear testing—that principle is embodied in the 1963 Partial Test Ban Treaty, and in the Nuclear Non-Proliferation Treaty—but since this treaty doesn't even come close to such a measure, Fisher argued last week that it could eventually torpedo the non-proliferation treaty (NPT). "It is silly to try to get other countries to sign the NPT while the nuclear powers are continuing to test and improve their own weapons", he said.

As for the provision limiting peaceful nuclear explosions, it was negotiated as a separate entity at the insistence of the USSR, which has plans for using nuclear blasts to divert rivers and for other excavation projects in Siberia. When the 1974 treaty was negotiated, the Soviet delegation wanted no limits on peaceful explosions, but the United States eventually insisted that such an agreement would leave a gaping loophole in the threshold test ban, since it would allow large-yield weapons to be tested under the guise of peaceful explosions. The United States, it should be noted, has virtually abandoned its own peaceful nuclear explosives programme.

At least the agreement now puts an upper limit on individual peaceful blasts, but it has nevertheless run into considerable opposition for treating peaceful explosions differently from weapons tests. The FAS statement, for example, notes that "this can only encourage new nuclear powers to justify bombs as intended for peaceful uses", just as India justified its detonation of a nuclear device. Fisher took the same tack: "we could end up with 20 nuclear nations all saying they are peaceful", he argued.

The FAS also regards the inclusion of provisions relating specifically to peaceful devices as an unwelcome prop underneath the 150 kiloton limit. "The linkage between peaceful and military uses makes doubly unlikely any reduction of the 150 kiloton ceiling. After all, such reductions would now require the Russians to reduce their peaceful limit as well", the FAS argues.

One much publicised aspect of the agreement on peaceful blasts is that, for the first time, it contains a provision for on-site inspection. Though the details of what would trigger such inspection, and what it would entail, have not yet been made public, the provision has been termed a major advance in negotiations with the USSR. Thus, Dr Fred Ikle, Director of ACDA, has said that "we consider this as a real breakthrough, from the point of view of arms control, to have been able to negotiate with the Soviets detailed arrangements for on-site verification".

But not everybody agrees. The FAS statement calls on-site inspection "a precedent whose time has passed" since seismic detection methods coupled with normal intelligence gathering "have narrowed, virtually to equivalence, the tests which can be detected but not identified". A source in ACDA also noted that on-site inspection would be limited to non-military regions in the USSR; extension of the concept to arms controls agreements involving military installations is entirely another matter.

Scoville, Fisher and the FAS have all argued that, instead of ratifying this treaty, the Senate should insist that the Administration should return to the negotiation table and come back with a treaty banning all nuclear testing. In a telephone interview last week, Fisher suggested that "in a couple of years, we should be able to negotiate a comprehensive treaty. We haven't really tried so far—and that goes for past Administrations as well".

At present, it is unclear whether opposition to the treaty, which is only just beginning to emerge, will be sufficiently strong to persuade the Senate not to ratify the measure. A check last week with staff people in the offices of several key Senators indicated that so far the matter has not been given close attention, and that few formal statements will be made until the full text of the agreement on peaceful explosions is made available.

It should be noted, however, that ratification will require a two-thirds majority vote in the Senate; in 1973, 30 out of 100 Senators co-sponsored a resolution urging the Administration to negotiate a complete, rather than a threshold, treaty. Senator Kennedy, moreover, has also denounced the terms of the 1974 agreement, which have been left essentially unchanged.

IN BRIEF

Japan's NPT difficulty

The Lower House of the Japanese Diet (Parliament) ratified the Non-Proliferation Treaty last week, but a Japanese nuclear arms ban along treaty lines may still be some way off. Powerful right wing opposition in the Upper House, which must approve the Bill, could see the Bill abandoned when the present Parliamentary session ends on May 24, and there seems little chance that the ratification can remain "on the table" for the necessary 30 days for it automatically to become law, since the crucial decision to extend the session by six days also rests with the Upper House. Hawkish opposition has constantly delayed ratification since Japan signed the treaty six years ago, even though it has passed through the Lower House before.

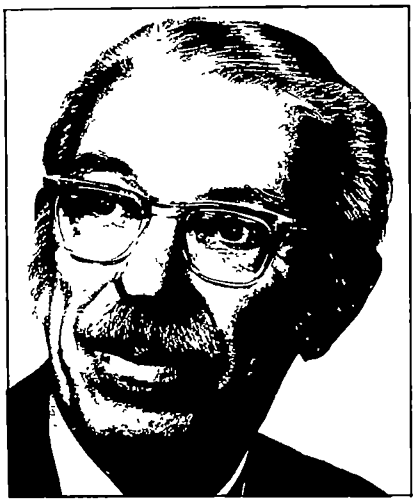
Cancer Institute resignation

In a development which could prove very embarrassing to the US National Cancer Institute, a top official in charge of the institute's environmental carcinogenesis programmes resigned last week, charging that his department is starved of manpower and subsumed under layers of bureaucracy. The official, Dr Umberto Saffiotti, is an outspoken individual who has persistently taken strong stands on the need to remove suspected carcinogens from the environment. His resignation is likely to fuel the growing political debate about whether or not the war on cancer in the United States is paying sufficient attention to cancer prevention.

EEC environment research programme

A five-year research and development programme aimed at introducing better control over the environment and environmental pollution has been agreed by Ministers of the EEC Nine, and to get the £6.7 millions programme under way the Commission has asked for specific research projects from Community interests concerned with most aspects of environmental pollution, reclamation, and ecological problems raised by modern methods of land use. In most cases, successful applicants will share research costs with the community. Laboratories from two UK ministries — Environment, and Health and Social Security—are expected to submit applications.

Federation meetings



THOMAS H. JUKES

THE Federation of American Societies for Experimental Biology includes the major American professional societies of physiologists, biochemists, pharmacologists, pathologists, nutritionists and immunologists. This April, the annual Federation meeting was in Anaheim, California, a short walk from Disneyland. I now wish I had completed the project I thought of starting about ten years ago.

I noted then that registration at the meetings had climbed to more than 15,000, but there didn't appear to be any increase in attendance at many of the numerous scientific sessions. Some were packed, but other meeting rooms were almost empty. Where was everybody? Quite a few were at the exhibits, especially at the free Coca-Cola booth, but it seemed to me that thousands were unaccounted for. I resolved to make head counts systematically in the scientific sessions and compare them each year with the numbers registered, but I got diverted, and in any case, the Coca-Cola booth has been replaced by vending machines. I wondered about the effect of Disneyland this year as a counter-attraction to science, but I was too parsimonious to investigate the matter.

The meetings are a tribal ceremony with a rich folklore. A decent respect for my contemporaries, and a fear of reprisals, prevents me from describing the 1940 Federation meetings in New Orleans. There was a fine scientific programme, and in those lean days a front seat at the floor show in the lively Vieux Carré night clubs could be had for the price of a bottle of beer. Details may be furnished by the President of the National Academy of Sciences, USA, if you can catch him in a reminiscent mood. He was there and it seemed to me that he didn't miss much.

After World War II, the societies grew so large that meetings were often held in the spacious accommodations at Atlantic City. Scientists seemed more serious in those years, and diversions in Atlantic City in April are mostly unappealing. One event stands out. Convention Hall in Atlantic City is designed for major political conventions, at which a band plays on a stage in the main auditorium. The stage is skilfully constructed so that the chairman can press a button and the stage, with the band playing diminuendo, is gradually lowered into the basement by an elaborate system of electric motors and reducing gears. The Candidate, Democrat or Republican, is then introduced to the ecstatic and partisan audience.

At the 1963 Federation meeting there was no band, of course. On stage was an eminent biochemist, Marianne Grunberg-Manago, to give an invited lecture on polynucleotide phosphorylase. Chairing the symposium was the urbane and famous Severo Ochoa, who graciously introduced Marianne. The first slide, showing how nucleoside diphosphates would form RNA-like polymers plus inorganic phosphate, came on the giant screen. The capacity audience was hushed and attentive. The chairman pressed a conveniently-placed button to dim the lights of the auditorium. Slowly and inexorably, platform, chairman, lectern and speaker descended out of sight.

Prominent scientific individualists of a type now unknown used to tread the boards at the meetings. Professor Barnett Sure had a strong dislike of the 15-minute time limitation imposed on regular papers. He would go on talking through the various warning lights and buzzers, through the discussion period, until he found the session chairman tugging at his sleeve. He would then glare at the chairman and say "First slide, please!" Many slides followed.

The American Society of Biological Chemists in 1976 decided to avoid the Federation Meetings, and are meeting separately, in June, at San Francisco, thus rejecting Mickey Mouse in favour of the adult entertainment for which San Francisco is famous. Watch for the report in *Nature*.

correspondence

Swine influenza

Sir,—The recent description of a human outbreak of influenza virus in an American military camp has justifiably given rise to a great deal of concern. The possibility that the virus involved might cause a severe pandemic must be seriously considered and it is perfectly understandable that vigorous measures be taken to deal with this contingency. Of such measures, the most effective is vaccination with the swine virus and the technology and resources at present available should make possible the production of sufficient quantities of inactivated vaccine for extensive use before the onset of a possible pandemic is anticipated.

Use of such a vaccine on a large scale, however, poses several problems apart from the cost of vaccine production and administration. In the first place, it may be difficult to justify the use of this vaccine for the protection of high risk subjects for whom vaccination is usually recommended, as the majority of such subjects belong to age groups in which antibody (and presumably resistance) to the swine virus is found in a high proportion of individuals due to exposure during 1918 and subsequent years of prevalence of this virus in human populations. Vaccination of younger age groups would be more justified especially considering the possibility of high mortality among young subjects as in the 1918 pandemic. It is difficult, not to say impossible, to estimate the probability of epidemic or pandemic spread of the swine virus.

This uncertainty should not dampen our efforts towards control, but what is to be our future action in the not too unlikely event of epidemics not being observed in the coming season? The risk will still be there in succeeding years and to ignore it will be unwise. If presently feasible inactivated virus vaccine is to be relied upon, the relatively short-lived protection it confers would lead to the daunting prospect of repeated early vaccination against a virus which might not appear at all. A more sensible solution would be to stockpile a strategic reserve of vaccine. Surveillance programmes in operation at present should be more than adequate to detect early signs of epidemic activity of the virus in time to enable the effective distribution and administration of vaccine. Adoption of this policy would make economic sense as the initial investment would be compensated by savings in avoiding the unnecessary use of vaccine in non-epidemic years.

If a strategic reserve of vaccine is acceptable as a potential defence against foot-and-mouth disease of cattle, this would be preferable to the possibly superfluous use of vaccine on the massive scale anticipated in the USA or to the vaccination of already resistant subjects as proposed in this country.

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Alternative refrigerants

Sir,—F. A. Cotton's letter (Correspondence, March 25, page 280) is an oversimplification although not, perhaps, a serious one. The fluorocarbon manufacturers estimate that the mean life of a fluorocarbon between manufacture and release to the atmosphere is about six months for an aerosol propellant and about two years for a refrigerant. The latter statement is surprising but is perhaps explained by the fact that a proportion of refrigerants is used in freeze drying and released immediately to the atmosphere, and also that in North America, at any rate, a considerable quantity is used in the air conditioning systems of cars which, perhaps sooner rather than later, are involved in head to tail collisions and release their fluorocarbons as a result.

Birks and Leck (Correspondence March 4, page 8) omitted to state that already in North America about 50% of total refrigerant used is refrigerant 22 which is unsuitable as an aerosol propellant This contains a carbon-hydrogen bond and, for reasons not completely understood but probably because this bond is destroyed by OH radicals which it can encounter in the lower atmosphere, its lifetime in the atmosphere is rather short, probably around three years, as compared with the 80–100 year life of refrigerant 12. It thus appears that refrigerant 22 is unlikely to cause a problem as far as stratospheric ozone is concerned.

The reason commonly given by the chemical manufacturers for not using refrigerant 22 exclusively is that this would leave manufacturing capacity unused as it is not possible to manufacture R-22 in a plant designed for R-12 However, I have been informed by a particular manufacturer that he routinely switches his plants from R-22 to R-12 according to demand. I was not previously aware that an R-22 refrigerant plant was much more expensive but, if this is so, it is difficult to understand why already half the refrigerant used is R-22.

There thus seems to be no valid reason why we should not dispense with the expensive, trivial and possibly dangerous convenience of the aerosol spray can—remembering that fluorocarbons are used almost exclusively in "personal care" products such as hair sprays and deodorants—and still retain the benefits of refrigeration.

W. J. Megaw

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Human anatomy

Sir—Kenneth Mellanby (February 19, page 523) remarked that model was a term capable of abuse, and that in Soho the word probably would not lead to useful scientific research. A week or two earlier the asymmetry of the scrotal sac of male homo sapiens had been discussed using artistic models as criteria.

Both these publications reveal the curious reluctance of scientists to research into human anatomy and relate it to function, disease and sociology. Such studies might usefully be termed gross morphological enquiries, in contrast with the vague constitutional studies which had a short flowering in the late nineteenth century and mid-twentieth century

I have found, for instance, that asymmetry is commonplace in the human physique. The circumference ratios (right/left) of thirty right-handed women in the 18–22 years age group were measured, and averaged 1.02 (ankle), 1.06 (calf), 1 05 (thigh), 1.09 (biceps). The volume of the mammary glands also differs, although supportive garments do not allow for this fact; the result is that the right mammary gland, if supported well, implies a looser support for the left. The explanation of these asymmetries is presumably in the greater use of the muscles underlying the right hand limbs.

No evidence for this asymmetry can be found in the paintings of Titian or Rubens in the National Gallery, London or in Rodin's The Kiss.

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news and views

Revival of Milankovitch

from George J. Kukla

To support a century-old controversial theory that no one has yet been able to prove or disprove convincingly is an unrewarding business. Obviously though, something great must be in the making if the head of the UK Meteorological Office, certainly not one to suspect of holding irresponsible revolutionary views, ardently re-approves the Milankovitch theory of glaciations (see *Nature,* **260,** 396: 1976). According to the report, Mason was impressed by the coincidence in the length of orbital periodicities with those found by Hays, Imbrie, and Shackleton (unpublished) in the ^{18}O records of some deep-sea cores. Geologists will be pleased but hardly surprised by the convincing answers of the computerised correlation. Fifty years ago, Eberl (*Die Eiszeitenfolge im Nordlichen Alpenvorlande,* 1930) demonstrated that the number of alpine Pleistocene terraces coincides with Milankovitch minima and 10 years ago, Broeker, (*Science,* **151,** 299; 1966) summarised available isotopic chronologies of Late Pleistocene and reported a remarkable resemblance to insolation curves. As for the energy involved, Mason sees the quantities in the "right ball park" which contradicts a view held by Shaw and Donn (*Science,* **162,** 1270; 1968). Now Weertman in this issue of *Nature* (page 17) demonstrates that Milankovitch variations are large enough to set the continental ice sheets into motion. But this is possible only if precipitation in high latitudes is much greater than today.

Here is where the problem begins: to find the details of the mechanism through which insolation changes climate. Although today the astronomical part of the Ice Age theory is fairly reliable, Koppen and Wegener's original ideas on the direct impact of insolation on glaciers are, in the light of modern climatology, naive at best. Redistribution of energy in the Earth–atmosphere system, which operates through a number of delicate feedbacks makes the problem extremely complicated (Kellogg and Schneider, **186,** 1163; 1974).

The largest effect of orbital perturbations is in the changes of seasonal and geographical distribution of radiation over the globe. In order to influence the climate, sensitive areas and seasons must be affected by insolation to a greater degree than in the rest of the year (Kukla, *Nature,* **253,** 600; 1975). Finding these zones and intervals is critical for understanding climate dynamics. No valid explanation of past climates or successful seasonal forecast is possible without first solving this key problem. Subdivision of yearly insolation into only summer and winter segments or simple global circulation models incapable of handling subtle feedbacks, can hardly provide an answer.

Suarez and Held (of Princeton University's Geophysical Fluid Dynamic Laboratory) used an energy balanced model which simulates the seasonal cycle and produces a large change in the extent of permanent snow and ice when past insolation structures are applied.

Berger (*EOS,* **57,** 254; 1976) has provided us with insolation values at twelve mid-month intervals in ten degree latitudinal belts for the past million years. His calculations of August irradiation on top of the atmosphere indicate a peak (744 Ly d^{-1}) 8,000 years ago at 60°N and a minimum of 679 Ly d^{-1}, 19,000 years ago. The difference of 65 Ly d^{-1} is undoubtedly large enough to affect the surface heat balance even without the volcanic dust triggers proposed by Bray (*Nature,* **260,** 414; 1976). On the other hand, the glacial–interglacial difference of 65 Ly d^{-1} is relatively small compared with the annual amplitude of irradiation to 60°N latitude. Insolation drops and rises by that amount in less than 10 days every October and April.

Despite millions of data bits on every element of the Earth–atmosphere system we still know lamentably little on the weather system response to this 10 day insolation shift. Can we expect to find the answers from spotty geological evidence accompanying the same irradiation change occurring over ten thousand years? Can we hope to understand glaciation and deglaciation without understanding fall and spring? With the wealth of data already at hand shouldn't we start to look seriously into the mechanism of the seasonal cycle? ☐

Control of transcription of the ovalbumin gene

from Pamela Hamlyn

THE well-characterised eukaryotic mRNAs have all been isolated from cells in which they are the predominant mRNA. The mRNA for ovalbumin is no exception being the major mRNA species in part of the hen oviduct, however, it is of particular interest since its production can be induced in immature chicks by treatment with the hormone oestrogen. On administration of the hormone there is a concomitant proliferation of tubular gland cells in the oviduct. When the treatment ceases (withdrawal) the number of these cells drops to 10–15% and ovalbumin synthesis becomes undetectable. This system is of interest to endocrinologists and also to molecular biologists, who have used it as a model system to study the control of protein synthesis (Palmiter, *Cell,* **4,** 189; 1975).

It has been known for some time that oestrogen, after diffusing into cells, combines with a protein, and that the complex migrates to the nucleus where it remains bound to the chromatin This is a specific reaction; it has been shown that the hormone–receptor complex will not bind to the chromatin of non-target cells Since the oestrogen appears to bind (by way of the receptor) to the genetic material, and since one of the observed effects is the synthesis of the protein ovalbumin, the study of the mRNA was an obvious next step.

Initially ovalbumin mRNA was

detected in RNA preparations by its ability to direct the synthesis of ovalbumin in cell-free protein synthesis systems. This method has now been superseded, however, by preparing a 'probe' for ovalbumin mRNA by transcribing the messenger with reverse transcriptase to obtain a radioactively-labelled complementary DNA. The cDNA is used to measure the concentration of mRNA sequences in immature chick oviduct cells before, during and after stimulation with oestrogen (Harris, Rosen, Means and O'Malley, *Biochemistry*, **14**, 2072; 1975). Before stimulation, ovalbumin mRNA was undetectable in oviduct cells, whereas several days' administration of oestrogen resulted in tubular gland cell formation and a build-up of ovalbumin mRNA to approximately 50,000 molecules per cell. Ovalbumin mRNA synthesis stops when the hormone is withdrawn and within 12 days the level of mRNA has dropped to 0-10 molecules per cell. These results have been used to suggest that the primary regulation of ovalbumin synthesis by oestrogen occurs at the level of mRNA transcription, that is, that the oestrogen–receptor complex on binding to the chromatin activates or 'derepresses' the ovalbumin gene so that it can be transcribed Other interpretations of these data are possible however. Cox *et al.*, for example, have pointed out the possibility that the effect of oestrogen could be to prevent the very rapid degradation of ovalbumin mRNA (Cox, Haines and Emtage, *Eur. J. Biochem.*, **49**, 225; 1974).

To investigate further whether the oestrogen–receptor complex has a direct effect on transcription, O'Malley's group have measured the number of RNA polymerase initiation sites on chromatin isolated from oviduct nuclei at various stages of oestrogen treatment (Tsai *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 4228; 1975). They have shown that during primary stimulation the number of RNA polymerase initiation sites increases, whereas withdrawal results in a drop in the number of initiation sites.

And now O'Malley and his coworkers have asked whether the RNA synthesised *in vitro* from isolated chromatin has the same tissue specificity as the RNA isolated from whole cells over the range of ovalbumin stimulation conditions. Using a cDNA probe for ovalbumin mRNA they were able to show that in their *in vitro* system only RNA transcribed from chromatin isolated from ovalbumin target cells contained ovalbumin mRNA sequences. They have also shown that RNA polymerase was necessary to produce RNA to which the cDNA would hybridise. (This is the usual control to eliminate the possibility that ovalbumin mRNA in the cell has been isolated as a contaminant in the chromatin preparation) Their results indicate that in general the presence of sequences transcribed *in vitro* which contain ovalbumin mRNA mimics the pattern of *in vitro* transcribed RNA with respect to hormone dependence The authors do point out one difference however During withdrawal there are between 0–10 molecules of ovalbumin mRNA per tubular gland cell but the chromatin isolated from the oviduct of withdrawn chicks could support the transcription of up to 5,000 molecules per cell. The authors suggest that the cells may fail to do this *in vivo* because of deficiencies in the RNA polymerase (or associated factors). Alternatively it may be (as Cox *et al.* also suggest) that low levels of mRNA are produced but are rapidly destroyed in the absence of post-transcriptional stabilisation which is in some way brought about by oestrogen.

O'Malley and his colleagues plan to use this *in vitro* system—to separate it into its components and reconstitute it —in order to study more directly how the oestrogen receptor interacts with chromatin. The continuation of this series (this latest paper is number eleven) will be awaited with interest.

Jovian magnetotail stretches past Saturn

from John Gribbin

In one of those surprising discoveries that, with hindsight, should not have been a surprise at all the Pioneer 10 sensors have detected the influence of Jupiter's magnetotail some 690 million km behind the planet, beyond the orbit of Saturn. This is just about the same distance from Jupiter as the Sun is on the other side—but since the magnetosphere surrounding Jupiter has a diameter of more than 14 million km it is not so surprising that the magnetotail stretches so far—about 50 magnetospheric diameters. After all, the Earth's magnetotail has been detected 500 planetary diameters 'downstream'.

Pioneer 10 passed through this extended magnetotail in mid-March, taking a full 24 h to complete its passage through the region of space influenced by Jupiter's magnetic field, according to NASA reports. Having passed the orbit of Saturn early in February, Pioneer 10 is now well on its way out of the Solar System, while its sister craft, Pioneer 11, re-targeted by its swing past Jupiter, is crossing the Solar System to encounter Saturn in three years time The evidence for the presence of Jupiter's magnetotail beyond Saturn's orbit comes from the Pioneer 10 solar wind detector, which registered zero during the time that the Jovian field 'shut out' the solar wind; the possibility that a genuine lull in the solar wind coincided with the spacecraft being exactly in Jupiter's magnetic shadow remains, but hardly looks plausible.

On the face of things, the observations imply that there is a roughly cylindrical volume of space influenced by Jupiter's magnetic field stretching across a sizeable fraction of the Solar System, and probably expanding slightly at greater distances from the Sun, since the solar wind density decreases as the square of the distance from the Sun. One of the most intriguing implications is that Saturn itself must pass through Jupiter's magnetotail every 20 yr, the next occasion being in April 1981 (unfortunately, just about 18 months after Pioneer 11 reaches Saturn). John Wolfe, Pioneer project scientist at NASA's Ames Research Center, comments that "this should produce some interesting magnetic phenomena".

One puzzle is that Pioneer 10 is now six degrees above the plane of Jupiter's orbit, and if the solar wind blows radially the magnetotail should be a couple of degrees, at least, below the spacecraft. But as earlier data from both Pioneer 10 and Pioneer 11 have shown, the solar wind blows gustily even at the distance of Saturn, and this turbulence could explain the tail being blown 'upwards' by six degrees temporarily.

The continuing irony of the new discoveries about Jupiter is, of course, the way in which space probes have shown that in many ways the most similar planet to the Earth is not Venus or Mars but the giant of the Solar System, a planet which contains three-quarters of all the Solar System's planetary material and which just missed, on some theories, becoming a star. ☐

Nations and numbers, 1975

from Robert M. May

For the past few years, the Environmental Fund in Washington DC has published an annual chart giving a country-by-country summary of the best contemporary estimates of population magnitudes, growth rates, and other demographic parameters. Some of these figures are familiar to most people, and some are not

Because of the many sources of uncertainty and delay in obtaining and reducing census data, the figures for

1975 do not simply derive from 1975 censuses. Rather they are extrapolations from the 1973 UN *Demographic Yearbook,* itself based on data up to 10 years old; the Environmental Fund's projections forward from 1973 derive from actual growth in numbers of people (from Table 4 of the 1973 UN *Demographic Yearbook*), rather than the more theoretically derived birth and death rates given in the latest UN estimates (April 1, 1975). The most significant uncertainty is in the figures for China.

In mid-1975, the total world population stood at around 4,147 million, growing at an annual rate of 2.2%. The dominant contribution was from Asia, whose 45 countries contributed 2,407 million people, with an overall growth rate of 2.5%; the growth rates of individual countries were scattered about this weighted average rate with a standard deviation of about ±0.7%. Other major geographical regions are Africa with 50 countries, 420 million people, growth rate 2.8%; and Latin America with 30 countries, 328 million people, and growth rate of 2.9%. The more developed regions are the 27 European countries, comprising 474 million people with an overall average growth rate of 0.8%; North America with 242 million and growth rate 1.0%; the USSR with 254, and 0.9%; and Oceania (comprised mainly of Australia) with 21 million people growing at 2.1% per annum. In each of these major regions, the growth rates of individual countries are scattered around the weighted mean value with standard deviations in the range 0.6–0.7; thus European rates span a range from −0.4 to 2.6, Latin American from 1.0 to 3.5, African from 1.2 to 3.8.

Of the larger countries (and henceforth I shall use "larger" to mean in excess of 10 million inhabitants), the top 1975 growth rate was Pakistan, at 3.6%. Despite its tribulations, South Vietnam was a close runner-up at 3.5, followed by Iraq and Kenya with 3.4%. In the open division of this event, regardless of the country's population, the clear winner was Kuwait with 4.8% (produced in part by immigration), from Libya on 3.8 and Rhodesia on 3.7. At the other end of the scale was East Germany with a negative population growth rate of −0.4%, followed by Portugal with −0.3, Hungary with 0.1, and Sweden and the United Kingdom on 0.2.

These different growth rates, past and present, make for great sociological differences in the countries. One important index of this is the fraction of the population which is under 15 years of age. In the world as a whole, 37% of people are under 15. By region, this breaks down into 44% in Africa, 43 in Latin America, and 40 in Asia, in systematic contrast with 26% in Europe, 27 in North America, 29 in the USSR, and 33 in Oceania. The top numbers are held by Iraq and Mexico, with a staggering 48% of the population below the age of 15; a dozen other countries (mainly in Latin America) follow with 47%. At the opposite end, Hungary has 20%, and East Germany and Sweden 21%. This ratio, which may crudely be thought of as a ratio of "tax consumers" to "tax producers", has a multitude of obvious implications for the social organisation of a country, some of which have been explored by Watt (*Principles of Environmental Science,* McGraw-Hill, New York, 1974) and others.

Maudlin (*Studies in Family Planning*, **6**, 30–36; 1975) has catalogued the developing countries according to the official government position regarding population growth or support of family planning. It is encouraging that 34 of these 118 countries, accounting for 2,083 million people (or 74% of the total among developing countries), have an announced policy to reduce population growth rates. In the case of India, the policy reaches back to 1952; 23 others date from the 1960s, and the remaining 10 from the 1970s. The average 1975 rate of population growth in this first category of developing countries was 2.6%. A second list of 32 countries (total population 454 million, representing 16% of people in developing countries) have official support for some form of family planning activities for reasons other than reducing population growth. The average growth rate in this category of "less alarmed" countries is 2.8%. A third category, including 52 countries (although only 270 million people, or 10% of those in developing countries) does not even have support for family planning.

It is notable that so large a fraction of the developing countries has adopted policies aimed at reducing

Natural selection and evolution

KREBS and May's account of social insects and the evolution of altruism (*Nature,* **260,** 9; 1976) is excellent, but the first paragraph raises a controversial issue. They attack statements of the type 'adaptations evolve because they are of benefit to the species (or population) as a whole'. They go on to point out, rightly, that natural selection acts on individuals, not on populations, but they then say: "Natural selection is a matter of differential survival and reproduction of individuals (or to be precise of genes), not of species". Evolution occurs over many generations, however, and genes do not exist in isolation but are part of gene complexes to which they must be adapted. Since most organisms reproduce sexually there will be many different gene complexes in the population over several generations. So for a gene to maintain its differential survival (that is, to increase its frequency or be selected) it must be adapted to many gene complexes, in other words to the entire gene pool of the population. This is what is meant by saying that an adaptation evolves because it benefits the population as a whole.

This point is important because a number of authors, particularly American ones, interpret 'adaptations benefitting populations' as implying group selection as defined by Wynne-Edwards (*Animal Dispersion in relation to Social Behaviour,* Oliver and Boyd, 1962). I have myself recently been criticised on these grounds (Dingle, *Science,* **188,** 1105; 1975), yet I never once mentioned group selection. There is an important distinction between natural selection, which relates to individuals or gene complexes, and evolution, which relates to changes in populations or in gene pools. When people write about adaptations evolving because they benefit the population or the species, this should not be thought to imply that they do not understand Darwinian natural selection, but rather that they are stressing the integration of the genes responsible for the adaptation into the gene pool.

MALCOLM EDMUNDS

JOHN KREBS REPLIES: Edmunds is correct in pointing out that good genes are ones which not only code for good characters, but also get on well with their fellow genes. However, selection does not act on large gene complexes, because they do not survive long enough as single units. Genes last a long time, so they are the units which survive in the process of natural selection. Where genes are very closely coadapted, they evolve very strong linkage so as to become one supergene.

Well adapted gene pools and gene complexes are obviously a consequence of natural selection for good genes, but this does not mean that gene pools and complexes are the basic unit of natural selection.

population growth. As stressed by Korten (*Studies in Family Planning*, **6**, 178–187; 1975), there are few other ideas in human history that have been transformed from taboo to official government policy in little more than a decade. Even so, the picture is not encouraging. The survey of aims and accomplishments with respect to population growth in the 14 largest developing countries (in order, China, India, Indonesia, Brazil, Nigeria, Bangladesh, Pakistan, Mexico, Philippines, Thailand, Turkey, Egypt, Iran, Korea) by Stewart (in *Population and the New Biology*, Academic Press, New York, 1974) reveals a consistent pattern of targets which, although modest enough, are not being met India's two-thirds cut-back in the 1974 budget for its family planning programme is the sort of economy it can least afford.

Another interesting demographic statistic is the percentage of a population that is classed as urban. This statistic is made a little fuzzy by virtue of the definition of an "urban" locality differing from country to country (see *Trends and Prospects in Urban and Rural Populations, 1950–2000*, UN, New York, 1975). Nonetheless, this figure shows interesting trends, particularly when the estimates for 1975 are put beside those for 1950.

In 1950, 29% of the world population was urban, as against 39% in 1975. Broken down into geographical groupings, these figures (expressed as percentage urban in 1950 and in 1975, respectively) are 23 and 44 for southwest Asia; 16 and 21 for middle south Asia, 13 and 22 for southeast Asia, 17 and 31 for east Asia; 13 and 25 for Africa; 41 and 60 for Latin America; 55 and 67 for Europe, 39 and 61 for the USSR; 64 and 77 for North America; and 65 and 72 for Oceania. Concealed within these aggregated numbers are such dramatic transformations as 28% urban growing to 63% over 25 years in Martinique, 11 to 37% in Zambia, 21 to 50% in Algeria, and many other examples of a more than 200% change in the urban fraction, particularly in Africa. Very little of this differential growth in urban populations is the result of planning. The absolute lowest urban fractions in 1975 come from Nepal (5%), followed by Tanzania (7) and Uganda (8). The most urban large country in both 1975 and 1950 was Australia (with 86 and 80%, respectively), slouch-hat jackeroo image to the contrary. □

Conservation of microbial genetic resources

from S. P. Lapage

The Third International Conference of Culture Collections (ICCC III), was held in Bombay, from March 15–19, 1976 and was attended by 290 scientists from 29 countries. It was organised by the World Federation of Culture Collections and local organising committee, under the auspices of UNEP, Unesco, ICRO and the UNEP/Unesco/ICRO panel, IUBS, The University of Bombay and other Indian organisations.

ICCC III provided a useful forum for discussion on the role and development of culture collections in developing countries. Emphasis was placed on the preservation of strains for local use and the preservation of those local strains which might otherwise be lost to science; on the relation of culture collections to the UN agencies and to the proposed development of regional microbial resource centres, with associated culture collections that would maintain the relevant strains of microorganisms, (for example suitable strains for nitrogen fixation or food fermentation in the particular region).

Also discussed were the value of national and regional federations of culture collections, the World Data Center and its relation to other data centres and their services in providing information on the location of the microbial genetic resources held in culture collections. The related problems of standardisation of data handling and of data coding for groups of microorganisms were also considered extensively, and it was thought necessary that the agreement of the relevant international authority for each group of microorganisms concerned (such as the International Committee of Systematic Bacteriology and International Committee on the Taxonomy of Viruses) be obtained before any system of data coding was universally recommended or adopted. The deposit of microorganisms for patent procedure in culture collections and an International Treaty proposed under the sponsorship of the World Intellectual Property Organisation were also discussed, as were the problems of identification in culture collections, and the value of culture collections in many other fields—medical, veterinary, industrial and others.

The technical aspects of preservation were also dealt with T W. G. Rowe (Edwards High Vacuum, Crawley, UK) announced some recent work of D. Greiff (Medical College of Wisconsin, Milwaukee) who has shown that ice and DMSO can be simultaneously sublimated under vacuum from a protein solution to leave a water-soluble 'cake', which opens up a prospect for the successful freeze-drying of microorganisms and possibly tissue cultures that do not survive freezing without a cryoprotectant. Rowe and Greiff have also shown the persistence of minute open channels in the heat sealed ends of Pyrex glass ampoules through which air and water vapour may percolate leading to deterioration of the contents. These channels can be closed simply by coating the sealed end with the synthetic rubber, neoprene. □

Drug action at the molecular level

from S. Neidle

A symposium on this topic was held on April 12–13 at the Middlesex Hospital Medical School, London, organised by the Co-ordinating Committee for Symposia on Drug Action, under the auspices of the Biological Council.

MOLECULAR pharmacology as an area of study encompasses a wide range of disciplines, from X-ray crystallography through membrane transport kinetics, to bio-organic chemistry. This diversity of approach was well illustrated at the recent symposium on drug action. Although the contributions covered a number of different problems, it became clear however, that many of these approaches are indeed to a large extent interdependent. Thus, "hard" results from say, kinetic studies concerning a biological receptor and the drugs which bind to it are best obtainable when the receptor itself has an established molecular structure. This point was exemplified by P J. Goodford (Wellcome Research Laboratory), who described recent attempts to design a drug to fit a known receptor. Haemoglobin, whose three-dimensional structure is well understood, was used as a model Diphosphoglycerate, a natural inhibitor of oxygen uptake by haemoglobin, fits into a geometrically well-defined binding cleft in the protein. Instead of synthesising series of diphosphoglycerate analogues, Goodford and his colleagues simply decided to manufacture a molecule that would fit exactly into the cleft, and have appropriate groupings to confer hydrogen-bonding donor and acceptor properties Biological testing of the inhibitor properties of their synthetic substrates have provided results which auger well for this novel approach.

The classical approach to systematic drug design was described by C. R. Ganellin (Smith, Kline and French). The simple concepts of structure–activity relationships, such as Hansch analysis using linear free-energy relationships, have been widely used. The limitations of these methods are now well known; however, Ganellin showed that dynamic structure–activity relationships are capable of analysing quite complex situations, such as relative ionic populations in a prototropic equilibrium mixture, and hence identifying the ionisation sites of the pharmacologically active tautomer of, say histamine. The relevance of this approach (and indeed of most others) to the identification of active conformers and tautomers was questioned by W. G. Richards (University of Oxford), who reported on his recent quantum mechanical calculations on histamine. He also emphasised the importance of considering the topography of both nuclei and electrons of drug and receptor in an active environment, in order to determine the essential conformation of the drug. The active conformation of histamine, as determined from conformational probability maps, seems to be neither that determined in the solid state by X-ray crystallography, nor that present in solution, and furthermore there is no obvious positive charge centre in the active molecule. Although these results have to be treated with caution, their implications are of some importance.

A hundred years ago

THE forty-seventh anniversary of the Zoological Society was held on Saturday last, Viscount Walden, F.R.S., the President, being in the chair. Mr. P. L. Sclater, F.R.S., the Secretary, read the report, which showed that the income (28,738*l*.) was greater than it had been in any previous year since the foundation of the Society. The total number of visitors in 1875 had been 699,918. The new lion house had been, as far as its main portions were concerned, completed and opened to the public. The building contains fourteen dens, the larger of which measure 20 ft. by 12 ft., the smaller being 12 ft. square. The out-door cages are to be completed by the end of July next; they will measure 44 ft. by 29 ft. Mr. Sclater desired it to be known that of the larger Felidae, the Ounce (*Felis uncia*) was a desideratum. The adoption of the report was moved by Prof. Huxley, seconded by Prof. Tennant, and carried unanimously.

from *Nature*, **14**, 15–16; May 5, 1876

Unsurprisingly, they provoked a lively discussion, during which some experimental support was suggested for essential conformations from structural studies on enzyme–substrate complexes. The audience, which consisted mostly of experimentalists, was doubtless relieved to hear that theoretical calculations of drug conformation are by no means infallible—indeed it is a characteristic experience that increasing sophistication in *ab initio* calculation often parallels increasingly poorer quality of results.

Magnetic resonance techniques for probing drug–receptor conformations in solution are currently enjoying a considerable vogue. Thus, J. Feeney (National Institute of Medical Research) showed how he has applied them to studies of hormonal peptides, such as oxytocin and gastrotetrapeptide. Although no direct information can be obtained about their conformations when bound to receptor sites, useful data indicating preferred modes of flexibility and intramolecular hydrogen-bonding organisation are more readily accessible. From this, Feeney and his associates have developed a "zipper" theory for the mechanism of receptor binding, whereby attachment is stepwise to a series of subsites, with each step giving a reorientation of the substrate conformation. G. C. K. Roberts (National Institute for Medical Research) presented a progress report on inhibitor bindings studied by NMR to the biosynthetically important enzyme dihydrofolate reductase. Although definitive conclusions must await an X-ray analysis, it is apparent that binding of, for example, the anti-leukaemic drug methotrexate (which is a competitive inhibitor for the natural substrate) is a complex process, which is accompanied by dynamic changes in the enzyme conformation.

Drug binding to nucleic acids, especially DNA, as receptors, has for long been a favoured field of study, doubtless partly because so much is known about the detailed structures of the nucleic acids. M. J. Waring (University of Cambridge) emphasised some of the challenging and unsolved problems in this area, particularly those concerned with binding to preferred lengths of sequence. Studies of binding with the antibiotic echinomycin, a cyclic octapeptide with two quinoxaline rings attached, have shown that this compound acts as a bifunctional intercalating agent. The two planar quinoxaline rings are believed to slot in between two base pairs, that is, with a gap of 10.2 Å. The problem of, *inter alia*, apparent violation of the neighbourhood exclusion principle at high binding levels has, however, hindered a detailed understanding of echinomycin binding to DNA so far.

It is not surprising that the complexity of membrane-bound receptor proteins has meant that knowledge of them at the molecular level is relatively sparse. Thus, the achievements of E. A. Barnard (State University of New York at Buffalo; now at Imperial College) and his associates in purifying the acetylcholine receptor (molecular weight 330,000) from denervated cat muscle, were the highlight of the session on these systems. It is worth remembering that the receptor, which was purified by binding to the snake venom α-bungarotoxin, exists in nanomolar concentrations in the tissue used. Results mentioned by Barnard from several laboratories have indicated the sites of receptor attachment to be the postjunctional membrane. The individual receptors are on the crest of the fold facing the presynaptic sites, and there appear to be roughly 20,000 individual molecules of protein per fold, arranged somehow within about 2,000 particles, as seen in freeze-etched electron micrographs. Clearly, this area of molecular pharmacology is destined for considerable advance in the near future. ☐

Solar system origin

from David W. Hughes

An advanced study institute on the Origin of the Solar System was held in the Institute of Lunar and Planetary Sciences of the University of Newcastle-upon-Tyne between March 29 and April 9, 1976 under the sponsorship of NATO.

COSMOGONY, the study of solar system origin processes is bedevilled by the fact that only one solar system is observable. The origin and evolution of stars (which are thousands of times further away and immeasurably less accessible than the planets) is much better understood simply because the sky is full of them—of different sizes and mass and at different stages in their evolution—and comparisons between them produce an abundance of clues to the physical processes responsible for their birth, life and death. The wavy paths of Barnard's star and Epsilon Eridani as they move across the celestial sphere indicate that they probably have planetary companions. Their remoteness, however, makes it completely impossible for us to measure the mass or orbits of the planets at present. An observer on one of the planetary companions of Epsilon Eridani, for example, looking towards the Sun would see Jupiter at greatest elongation 1.6″ away from the Sun and as an object 10^{10} times less bright. Jupiter's image would thus be completely swamped by the glare of the Sun. The centre of the Sun moving around the centre of mass of the Solar System would produce an astrometric wave of peak-to-peak amplitude 4×10^{-3}″ which is well below present-day detection limits. So a solar system such as ours orbiting a nearby star would be undetectable. The companions of Epsilon Eridani and Barnard's star must be much heavier and/or much closer to the central star than our Jupiter or Saturn.

Bearing these difficulties in mind a group of cosmogonists (a "nebulosity" of cosmogonists might be the better collective noun considering the scant common ground between their theories) met for two weeks at the University of Newcastle-upon-Tyne to discuss their latest theories and to explain them in detail to the many research workers in related fields who had also assembled there.

The best resumé of the problem was given by H. Reeves (CNRS, Saclay) who expanded on his recent paper (*La Recherche*, **6**, 807; 1975). Cosmogonic theories were divided into four groups dependent on whether the Solar System was formed at the same time or later than the Sun and on whether the Solar System was formed out of interstellar material which has the same chemical composition as present-day interstellar material or from material that has been altered chemically as a result of being at one time inside a star and being heated up to temperatures near 10^6 K. Observational facts are used to help decide between the above options.

The facts that the deuterium/hydrogen ratio is about 2×10^{-5} in planetary and interstellar material but less than 3×10^{-7} in the Sun, the lithium/silicon ratio is 200 times smaller in the Sun than in the planets, whereas the iron/silicon ratio is reasonably the same, indicate strongly that the Solar System is made of unaltered material, thus ruling out two of the options.

Ratios between the abundances of parents and decay products in radioactive series indicate that the Earth, Moon and most meteorites solidified about 4.6×10^9 yr ago The ^{12}C/^{13}C ratio has halved over the past 5×10^9 yr and measurements of this ratio in cometary gas and the atmosphere and surface rocks of planets indicate that the Solar System was formed quickly, and at the same time as the Sun. So the nebulosity, parts of which condensed to form the planets, was dense and short lived. The cosmochemistry of the Solar System, which accounts for the mean composition of the planets and its variations with heliocentric distance, again requires a massive nebula (~10% of the solar mass), opaque to radiation, with temperatures varying from 1,500 K at about 0.3 a.u. from the Sun to 200 K at 40 a.u., and pressures of between 10^{-3} and 10^{-6} atmospheres. This temperature variation accounts for the rocky nature of the terrestrial planets and the icy and gaseous nature of the outer planets. It must also be remembered that only about 1% of the original nebula finally coalesced into planets, 99% being lost from the system.

The Reeves scenario rules out all theories which call on cloud capture long after solar system formation, interstellar collisions and binary star origins and leaves one with the basic Laplace nebula which was first propounded in 1796. All other theories have to be carefully scrutinised in the light of the above observations.

A. G. W. Cameron (Harvard College Observatory) and H. Alfvén and G. Arrhenius (University of California) were protagonists in a fascinating debate starting from positions 5×10^9 yr apart. Cameron concentrated on star formation (that is, birth of the Solar System), invoking a pressure-induced collapse of an interstellar cloud caused by a recent encounter with a galactic spiral arm and a nearby supernova explosion. This cloud forms a cool, turbulent, dense disk out of which large protoplanets condense and subsequently disrupt leaving, as debris, the present planets. Magnetic fields and plasma effects play no part.

Alfvén and Arrhenius pledged allegiance to the 'actualistic' principle, the present state of the Solar System in conjunction with the physical processes now acting being progressively wound backwards so that older and older times are reconstructed. In the resulting theory the Solar System condenses slowly out of a low density nebula which is partially ionised and controlled by magnetohydrodynamic processes. At this stage in the debate problems of angular momentum raised their heads. These are that a one solar mass cloud of average galactic density (1–10 hydrogen atoms cm^{-3}) and differential rotation would, if it condensed to form a star, form a star which was spinning too quickly, was too hot and too highly magnetised; the planets contain 0.14% of the mass but 98% of the angular momentum of the Solar System; and conversely satellites of planets only contain 10–20% of the planet's angular momentum.

Alfvén transfers angular momentum from the Sun to the planets using Birkeland currents similar to those acting in the ionosphere and magnetosphere. Cameron's Sun is originally spinning quickly and is braked over the 5×10^9 yr by solar wind drag. D. C. Black (NASA–Ames Research Centre) makes use of the apparent rarity of star formation and the fact that stellar rotation axes are orientated randomly with respect to the galactic plane to conclude that gas clouds only collapse if the galactic corotational angular momentum is almost completely cancelled out by the turbulent angular momentum of the cloud. M. M. Woolfson (University of York) does not have this problem as in his theory the gas pulled from a protostellar cloud when the Sun passes close by has an independent angular momentum to the Sun's.

G. Herbig (Lick Observatory, California) reviewed observations of the young T Tauri type stars and indicated how their high shrinkage rates, large-scale mass ejections, variability and apparent nebula envelopes were of considerable relevance to planetary formation.

A. J. R. Prentice (Monash University, Australia) was the only contributor to introduce directly the Titius–Bodes law. His "Laplacian" theory predicted rings of planetismals at the presently observed heliocentric distances but this again leaves the problem of how and why these planetismals accrete to form one, and only one, planet from each ring. The accretion and fragmentation processes taking place between these planetismals were discussed in detail by A. W. Harris (Jet Propulsion Laboratory) and J. K. Kerridge (Institute of Geophysics, University of California). W. H. McCrea (University of Sussex) suggested that Earth–Moon–Mars and Venus–Mercury were just fission products from earlier larger planets.

Diverse ideas and suggestions were put forward by many people but the list of unanswered questions remains uncomfortably long. What do meteorites have to tell of the conditions in the nebula during the early Solar System? Opinions differed greatly as to their significance. Why didn't the Asteroids form a planet? Is the planarity and orbital directness of the present Solar System just the result of 4.6×10^9 yr of gravitational perturbation? Is there anything apart from comets beyond Pluto? Why did meteorites undergo a second heating after formation? Just how common are solar systems like our own? The remaining questions are legion, and the debate will go on far into the future; however, this study institute at Newcastle gave the problems a good airing and introduced many people to the fascinations of this intriguing subject. Two weeks of stimulating mental activity was had by all. □

articles

Milankovitch solar radiation variations and ice age ice sheet sizes

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The fluctuations in the size of ice age ice sheets are calculated using glacier mechanics and the Milankovitch solar radiation variations. The calculations are greatly simplified by considering only two-dimensional ice sheets with profiles that would be appropriate if ice obeyed the flow law of a perfectly plastic solid. The solar radiation variations seem to be large enough to account for ice ages.

The Milankovitch theory of the ice ages[1,2] is based on the long term variation in the solar radiation received at presumed critical northern latitudes during certain seasons of the year. This variation is produced by changes in the magnitude of the eccentricity of the Earth's elliptical orbit around the Sun, the precession of the Earth's axis, and by changes in the value of the tilt of the Earth's axis. The eccentricity varies between the limits of 0 and 0.06, with an average period of 93,000 yr; the angle of tilt of the Earth's axis varies between 22.1° and 24.5° with an average period of 41,000 yr; and the average, precessional period is 21,000 yr (ref. 1). Ice ages are assumed to occur when the total solar radiation for the half year that contains spring and summer at certain northern latitudes is at a deep minimum value. Interglacial periods are assumed to occur when the solar radiation has a high maximum value.

Figure 1 shows the variation, ΔQ, over the past 300,000 yr and the next 80,000 yr of the solar radiation, Q, averaged over the high radiation half of the year at 50°N (ref. 1). Today the value of Q, measured in langleys d^{-1} (1 langley (ly) = 41.85 kJ m^{-2}), at the latitudes of 60°N, 50°N, and 40°N is 787, 847, and 892 respectively for the high radiation half of the year[1]. Thus the variation ΔQ that is shown in Fig. 1, which is between the limits of $+50$ ly d^{-1} and -35 ly d^{-1}, is the same as that produced by shifting the land mass upon which an ice sheet may be resting southwards by up to 9.5° latitude (or $\sim$ 1,000 km) and northwards by up to 6.7° (or $\sim$ 750 km).

No attempt has been made so far to apply the theories of the mechanics of ice age ice sheets[3] or of the rates of growth and reduction of ice sheets[4] to determine whether the Milankovitch solar radiation variations are indeed large enough to produce the very large changes in the dimensions of ice age ice sheets. The half width L of these ice sheets varies with time between the limits of about $0 < L < 1{,}000$ km. Barry, Andrews, and Mahaffy[5] have attempted to calculate how fast a continental ice sheet nucleated in eastern Canada might form, but they made no comparison with the Milankovitch theory. Calder[6] calculated fluctuations of the volume of ice age ice sheets but did so with no consideration at all of glacier mechanics. He simply assumed that the rate of increase or decrease of the ice volume was proportional to the Milankovitch variation in radiation.

An attempt is made here to see if the Milankovitch solar radiation variations are sufficient to produce large variations in the size of ice age ice sheets.

The model

To make things simple I will consider a two-dimensional ice sheet that rests on a land surface that was flat before the ice sheet formed on it. Figure 2 shows the ice sheet in profile. The lower ice surface is not flat because of isostatic depression of the land surface. The northern edge of the ice sheet, at the border of the Arctic Ocean, is situated at $x = -L$ and the southern edge is at $x = L$. The total width of the ice sheet is therefore $2L$. The elevation of the upper ice surface above the original position of the land surface is $h(x)$ and the depression of the lower ice surface is $h'(x)$. When isostatic conditions prevail and the density of rock is taken to be three times that of ice, $h'(x) = \frac{1}{2}h(x)$. The total thickness of the ice is equal to $(3/2)h(x)$.

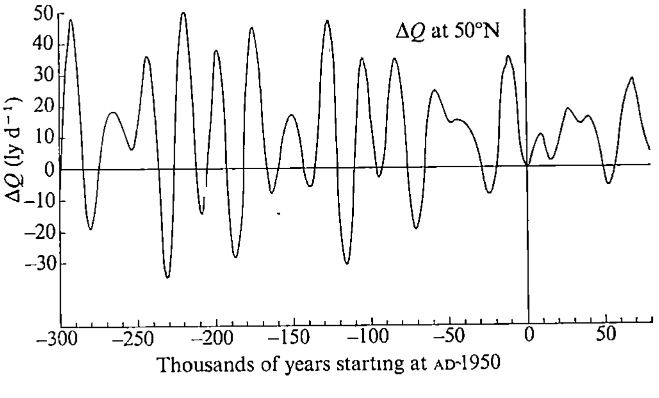
Let the ice sheet profile be approximated by that determined with the flow law of a perfectly plastic solid. The profile of an actively flowing ice sheet is given by the equation

$$h(x) = h(-x) = [\lambda(L-|x|)]^{1/2} \qquad (1)$$

Here $\lambda = 4\tau/3\rho g$, where τ is the yield stress which is equal to the basal shear stress ($\sim$ (0.5–1)$\times 10^5$ Pa)(1 Pa = 10^{-5} bar), ρ is the density of ice, and g is the gravitational acceleration. The value of the constant λ is $\sim$ 7–14 m.

To remain in existence the ice sheet must be nourished by snowfall. Let $h_s(x)$ represent the snowline elevation. At elevations on the ice sheet $h(x) > h_s(x)$ there is, over the year, a net accumulation of ice and at elevations $< h_s(x)$ there is a net

Fig. 1 Milankovitch radiation variation[1].



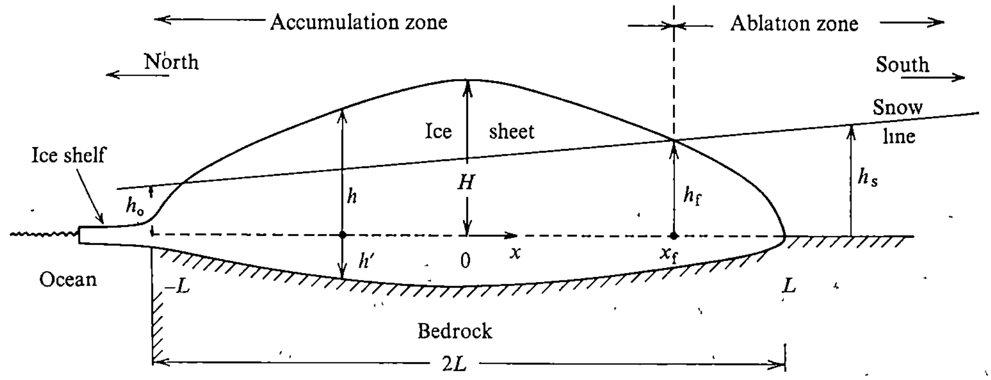


Fig. 2 Cross section of ice age ice sheet.

ablation of ice. Assume that the snowline elevation above the original land surface can be approximated by a simple linear relationship

$$h_s(x) = h_0 + s(x+L) \tag{2}$$

where s is the slope of the snowline and h_0 is the snowline elevation at the northern edge of the ice sheet. The snowline to be used in any calculation is the one that would be found by placing successively larger ice sheets on the land surface and observing on these ice sheets the elevation $h(x_f)$ at the firn line position x_f that separates the accumulation zone from the ablation zone (see Fig. 2). This snowline, which exists in the presence of an ice sheet, will be lower than the one that exists now.

Equilibrium ice sheet

All the ice accumulated on the northern half of the ice sheet will flow out in ice shelves into the ocean as ice today flows out of the Antarctic Ice Sheet. In the southern half of the ice sheet the ice gained in the accumulation zone eventually must be lost in the ablation zone. The average accumulation rate $\langle a \rangle$ over the whole southern half of the ice sheet is equal to

$$\langle a \rangle = [a x_f - a'(L - x_f)]/L \tag{3}$$

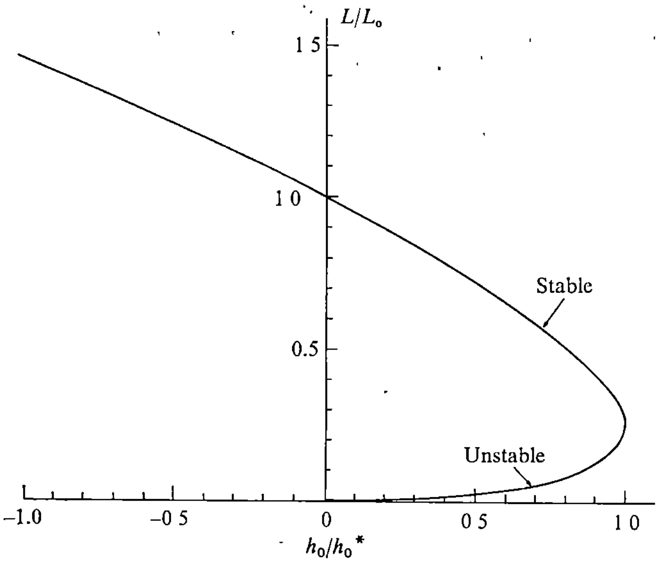
where a is the average accumulation rate within the accumulation zone and a' the average ablation rate in the ablation zone. Both a and a' are positive quantities.

Obviously an ice sheet is in equilibrium only if $\langle a \rangle = 0$. The half width L of an equilibrium ice sheet is found by setting $\langle a \rangle = 0$ in equation (3) and then combining that equation with equations (1) and (2). The result is

$$(L/L_0)^{1/2} = \tfrac{1}{2}[1 \pm (1 - h_0/h_0^*)^{1/2}] \tag{4}$$

where $L_0 = \lambda(1+a'/a)/s^2(1+2a'/a^2)$ and $h_0^* = \lambda/4s(1+2a'/a)$. Figure 3 shows a plot of L/L_0 against h_0/h_0^*. Obviously no equilibrium ice sheet can exist if $h_0 > h_0^*$. The plus sign in equation (4) gives the half width L of a stable equilibrium ice sheet and the minus sign the half width L of an unstable equilibrium ice sheet. If $h_0 < 0$, only the solution of the stable equilibrium ice sheet is physically meaningful. A stable equilibrium ice sheet over time approaches its equilibrium size if its dimensions are perturbed. An unstable equilibrium ice sheet either shrinks until it disappears or grows until it reaches the stable equilibrium size if its dimensions are perturbed. The change in size of a non-equilibrium ice sheet is a consequence of the fact that $\langle a \rangle \neq 0$.

Fig. 3 Normalised plot of equilibrium ice sheet size against normalised snowline elevation at northern edge.



It should be noted that the half width L of the equilibrium ice sheet is independent of the actual values of a and a'. It only depends on the ratio a'/a. This result is not valid if a power-law creep equation is used to determine the ice sheet profile. The resulting dependence of L on a and a' is, however, relatively weak[3].

The magnitude of terms L_0 and h_0^* should be noted. For the not unreasonable values $a'/a = 2.75$, $s = 10^{-3}$, and $\lambda = 14$ m one finds $L_0 = 1{,}245$ km and $h_0^* = 0.54$ km. Now suppose h_0 were to fluctuate in value between limits > 0.54 km and < 0 km because of Milankovitch variations in radiation. The value of the stable equilibrium half width L of the ice sheet would alternate between 0 km and a limit $> 1{,}245$ km.

Time variation of ice sheet size

The variation in time, t, of the volume, V, of the southern half of the ice sheet is simply

$$\mathrm{d}V/\mathrm{d}t = a x_f - a'(L - x_f) \tag{5}$$

For an active ice sheet $V = (\lambda L^3)^{1/2}$ and the rate of change $\mathrm{d}L/\mathrm{d}t$ is given by

$$\begin{aligned}\mathrm{d}L/\mathrm{d}t = (2a/3\lambda^{1/2}L^{1/2})\{L - [(1+a'/a)\lambda/4s^2] \times \\ \times [-1 + (1 + 4s[h_0 + 2sL]/\lambda)^{1/2}]^2\}\end{aligned} \tag{6}$$

This equation is found by combining the preceding equations. Since for an active ice sheet $h(x) = h(-x)$ the northern half of the ice sheet changes its size at the same rate as does the southern half.

Equation (6) applies only if the ice sheet is actively flowing, that is, the basal shear stress is equal to the yield stress. Now it could happen that the snowline is raised so that the whole ice sheet is in an ablation zone. In this situation the removal of ice reduces the basal shear stress to below the yield stress and ice no longer flows. The ice sheet is stagnant. The rate of wastage of the ice sheet can be quite rapid in this situation[4]. The rate of

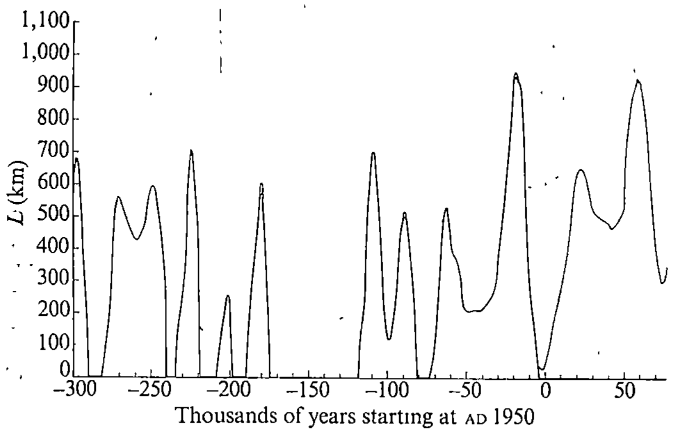


Fig. 4 Half width, L, of ice sheet as a function of time calculated with $\lambda = 14$ m, $\Delta Q_0 = 10$ ly d^{-1}, $s = 0.001$, $\alpha = 0.021$ km ly^{-1} d, $a = 1.2$ m yr^{-1}, $a'/a = 2.75$ (thick line) and 2.745 (thin line).

change dL/dt whenever $h_s(0) > h(0)$ will be approximated by the equation

$$dL/dt = -2a'(L/\lambda)^{1/2} \tag{7}$$

This rate of shrinkage is three times faster than that found by assuming that the ice sheet is active.

A growing ice sheet can also be stagnant. Suppose that the snowline is suddenly lowered and h_0 has a negative value. If $-h_0/s > 2L$, snow will accumulate on the ground ahead of the ice sheet, or an ice sheet will nucleate if $L = 0$. In this situation it will be assumed that either a is large enough or the term $-h_0/s$ is sufficiently small that no serious error is introduced by setting $L = -h_0/2s$ and $h(x)$ the value given by equation (1).

Connection with the Milankovitch radiation variation

We saw earlier that the Milankovitch variation in the solar radiation ΔQ is similar to that produced by shifting the land surface north and south by distances ~ 1,000 km. If the snowline were fixed in space but the land surface were shifted north and south by such distances the effect would be the same as keeping the land surface fixed and moving the snowline elevation up and down. In other words the value of h_0 would be given by the equation

$$h_0 = \alpha(\Delta Q + \Delta Q_0) \tag{8}$$

where ΔQ_0 is a constant and

$$\alpha = s/(dQ/dx) \simeq (21 \text{ km ly}^{-1}\text{d}) \times s.$$

We assume that in this naive way the solar radiation variations can be related to changes in the elevation of the snowline.

The Milankovitch radiation variations undoubtedly cause changes in the value of a, a', the ratio a'/a and possibly in the slope s. Only the change in size of the ice sheet produced by changes of the snowline elevation given by equation (8) will, however, be considered because there seems to be no good way of estimating how the other quantities are altered by the variations of the radiation.

I have calculated the variation of L using equations (6), (7) and (8) and the Vernekar tables[1]. The Vernekar tables list the value of ΔQ at 1,000-yr intervals over the past 2 Myr and for the next 120,000 yr. The computer calculations were made incrementally by calculating $\delta L/\delta t$ where δt was taken as 1,000 yr. The values of ΔQ chosen were for 50°N. The value of L 2 Myr ago was set equal to zero. (For Fig. 6 at 1 Myr ago.)

Figure 4 shows two plots of L over the past 300,000 yr and for the next 80,000 yr for $s = 10^{-3}$, $\lambda = 14$ m, and $a = 1.2$ m yr^{-1}. Two values were chosen for a': $2.75a$ and $2.745a$. The value of ΔQ_0 was taken to be 10 ly d^{-1}, which gives $h_0 = 0.2$ km at the present time. The value of h_0 over the past 300,000 yr varies between the limits of 1.3 km and −0.5 km. Figure 5 shows the same plot, for $a' = 2.745a$, carried back to 2 Myr and superimposed on the Milankovitch curve.

I believe that Figs 4 and 5 demonstrate that the Milankovitch radiation variations are potent enough to be the prime cause of ice ages, although these plots by no means prove that the Milankovitch ice age theory is correct. The magnitude of the highest peaks, ~ 1,000 km, is of the right order of magnitude. The position and relative magnitudes of the peaks and troughs over the past 130,000 yr correlate well with sea-level fluctuations over the same period (see Fig. 7 of ref. 7). The main criticism that can be directed at Figs 4 and 5 is the use of a rather large, but not impossibly large, accumulation rate as well as ablation rate. At the present time the precipitation in north-eastern Canada is about half this level.

If a much smaller ratio a'/a is used the value of h_0^* becomes so large that once an ice sheet is formed it never disappears.

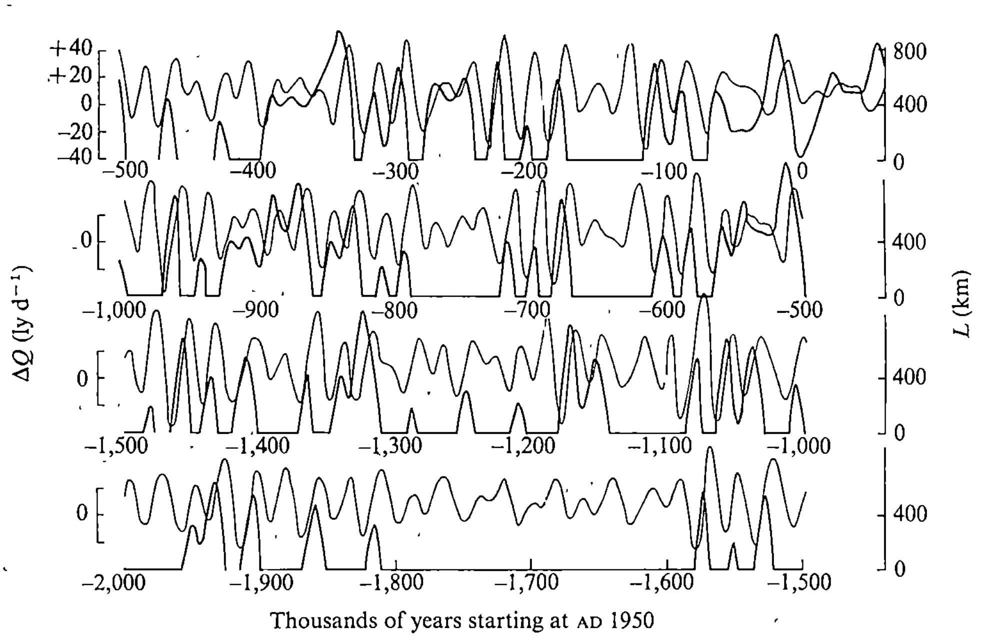


Fig. 5 Same plot as Fig. 4 for the case $a'/a = 2.745$ but for a longer time period. Also shown is the radiation variation.

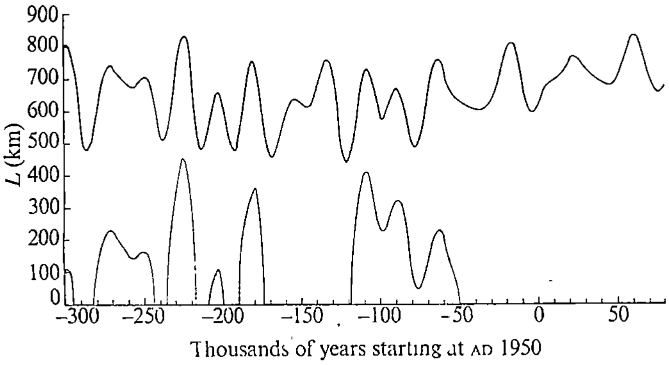


Fig. 6 Same plot as Fig. 4 but with $\lambda = 14$ m, $\Delta Q_0 = 10$ ly d^{-1}, $\alpha = 0.02625$, $a = 0.3$ m yr^{-1}, and $a'/a = 2.2$ (thick line) and 2.4 (thin line).

If a much larger ratio is used the ice sheet never grows to a large size. If smaller values of a as well as a' are chosen, the peak size of the ice sheet is reduced. Figure 6 shows an example of reduced peak size. This figure also illustrates a case of non-disappearance of an ice sheet. The use of $a = 0.6$ m yr^{-1} can produce peaks $\sim L = 700$ km.

The smaller value of a produces smaller peaks in L, not because the equilibrium size of the ice sheet is small, which it may not be, but because the rate of build up of the ice sheet is smaller. The ice sheet does not approach a large equilibrium size before the value of ΔQ changes and causes the ice sheet to waste away.

Figure 4 also demonstrates the hazard of using a Milankovitch curve to predict future ice ages as well as to determine when past ice ages must have occurred. According to Fig. 4 and data not plotted, depending on whether $a'/a = 2.750$ or $a'/a = 2.745$, there will be no ice age for the next 120,000 yr or one actually is starting to build up right now. (Note that the value of L for the thin line curve at $t = 0$ is $\sim L$ of existing ice caps in the Arctic.) By assuming different values of the constant ΔQ_0 new peaks can be made to appear in the plots or old peaks can be made to disappear.

Conclusion

I believe that it has been demonstrated that the solar radiation variations that are the basis of the Milankovitch theory are large enough to have produced ice ages. To make the large ice age ice sheets that are known to have occurred, however, it is necessary that high accumulation and ablation rates existed at least on the southern half of the ice sheets—rates that are substantially higher than occur today in Greenland and Antarctica. These calculations demonstrate the rather unusual position the northern land masses must have for it to be possible for ice ages to come and go over the past 1–2 Myr. If the northern land masses were all shifted southwards by $\sim$ 500 km it would be equivalent to increasing ΔQ by $\sim$ 25 ly d^{-1}. Suppose all other factors remained constant (precipitation is not changed, for example, in spite of the altered water circulation into and out of the Arctic Ocean). After such an increase in ΔQ it would be very difficult to give predictions for ice ages with large ice sheets using our model while at the same time requiring the model to predict alternating ice ages and interglacials with the land masses in their present position. Similarly if the land masses were shifted northwards by $\sim$ 500 km it would be difficult to make the model predict anything other than a perpetual ice age.

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Thermal radiation produced by the expansion of the Universe

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I show here that the expansion of the Universe can create particles with a black-body distribution. I give a simple model in which the creation of particles in an initially empty spacetime leads at very early times to a Friedmann expansion dominated by hot relativistic particles (probably including gravitons) having a black-body spectrum, even before classical thermalising processes have had a chance to act. If the particle creation occurs near the Planck time, the entropy produced is consistent, in order of magnitude, with that required by the Einstein equations. The conditions in which one achieves consistency with the observed 3K black-body radiation are also discussed.

THE model we discuss in detail here assumes a Robertson–Walker (isotropic) space time which expands from a possibly singular state characterised by the universal constants G, $\hbar$, and c. The metric is treated classically, whereas the matter and radiation are quantised. The equations of the quantised particle fields are the simplest (minimally coupled) generalisations of the special relativistic free-field equations. We assume that other interactions have a negligible effect on the particle creation process near the singularity, compared with gravity. No particles are present initially. Near the singularity, the particle field equations for spins other than 0 and 2 are conformally invariant (the masses can be neglected because of the high energies involved), so that they lead to no particle creation in the isotropic model[1–3]. Mesons obeying the minimally coupled spin-0 field equation will, however, be created in abundance near the singularity. We show that these mesons have the remarkable property that they are created with a black-body spectrum, and hence will be in thermal equilibrium *ab initio*, even in regions of space time which are not causally connected[4]. The same can be said of gravitons obeying the linearised Einstein equations given by Grishchuk[3], since each component $h_i{}^k$ obeys our equation (2) when the τ-time defined below is used. Entropy is generated because the particles are created in pairs, and correlations between members of a pair are unobservable on account of subsequent interactions and decays. Although these results do not make use of the Einstein equation which implies that the density at t_p is of the order of the Planck density, they are consistent with it[5,6], and further provide a mechanism for producing the necessary entropy. It should be noted that in the model considered here, one starts with very simple initial conditions (empty Minkowski space) and obtains a black-body spectrum at a very early time, independent of classical thermalising mechanisms. (Our results are not necessarily inconsistent with a 'bouncing' model, as in refs 6 or 7, if appropriate boundary conditions are imposed at the minimum of $a(t)$.)

Any mechanism consistent with the Einstein equations which yields uniform black-body radiation before t_e, the time at which the matter and radiation densities become equal, will lead to an expression for the present black-body temperature which depends on t_e raised to the one-sixth power. We interpret this expression as giving the present temperature to be approximately equal to the redshifted temperature of the initially created particles.

To obtain the present matter density, and thus t_e, from this model, one could use a matter–antimatter separation mechanism (possibilities are reviewed in ref. 19), or an interaction which did not conserve baryon number. One could also introduce the excess baryon number in the initial state, without significantly affecting the temperature. We make no attempt here to predict the entropy per baryon.

In anisotropic expansions with similar boundary conditions on the minimally coupled scalar field, we find that the particles are created with a direction dependent distribution resembling Doppler shifted radiation in an anisotropically expanding thermal cavity with perfectly smooth reflecting walls and no dust. As in the latter case, one would expect interactions to bring out a true black-body spectrum, while, as suggested by Zeldovich, the expansion itself was isotropised through the gravitational interaction of the created matter. Our results should be similar to those of ref. 8, the main modification being in the initial particle distribution. A special case of such a distribution was found by Berger[9] in the degenerate Kasner universe. We, however, view our present boundary conditions, incorporating $\hbar$, c and G, as corresponding to a physical process occurring only when the curvature is sufficiently large, and not near a pseudo-singularity, as in the degenerate Kasner.

From a fundamental viewpoint, the thermal production of particles may point towards a generalisation of the association of entropy and temperature with black-hole space times[10,11] to cosmological space times, or to singularities themselves. In that connection, the cosmological thermal emission may have a role analogous to the thermal emission by black holes discovered by Hawking[12].

The spectrum and probability distribution of the particles created by the isotropically expanding universe have been given before[1,2], but the detailed form of the coefficients $|\beta/\alpha|$ involved, and the connection with black-body radiation, is given here for the first time. More details, together with a treatment of the anisotropically expanding model, will be published elsewhere.

Isotropic model

For simplicity, we consider the spatially flat Robertson–Walker metric

$$ds^2 = dt^2 - a^2(t)\sum \delta_{ij} dx^i \, dx^j$$

The presence of spatial curvature will not significantly alter the result. As noted earlier, there is significant particle creation only by the minimally coupled spin-0 (and spin-2) field equation $\nabla_\mu \nabla^\mu \varphi = 0$, where ∇_μ denotes the covariant derivative. (We use units with $\hbar = c = 1$.) The mass can be neglected because the energy of the created particles which contribute to the present black-body spectrum is very large compared with elementary particle rest masses. Canonically quantising the field in the usual way, we write

$$\varphi(\mathbf{x},t) = (2\pi)^{-3/2} \int d^3k [A_{\mathbf{k}} e^{i\mathbf{k}\cdot\mathbf{x}} \psi_k(t) + \text{H.C.}] \qquad (1)$$

where $k = |\mathbf{k}|$, and the $A_{\mathbf{k}}$, $A_{\mathbf{k}}{}^\dagger$ obey the usual commutation relationships. For simplicity, we are considering the neutral field. In ref. 1 we showed that charged particles are also created (in particle–antiparticle pairs) with the same probability distribution. The functions $\psi_k(t)$ satisfy

$$d^2\psi_k/d\tau^2 + a^4 k^2 \psi_k = 0 \qquad (2)$$

with

$$\tau = \int^t a^{-3}(t')dt'$$

The Wronskian condition is $\psi_k(d\psi_k{}^*/d\tau) - \psi_k{}^* (d\psi_k/d\tau) = i$.

Boundary conditions

We assume that for t large with respect to t_p, the expansion parameter $a(t)$ is given by the general relativistic solution for a universe dominated by relativistic particles and radiation, namely $a(t) = Ct^{\frac{1}{2}}$, where C is a constant which does not enter our final results. As t approaches t_p, we let $a(t)$ join smoothly to a constant value a_1 given, approximately, by $a_1 \simeq Ct_p{}^{\frac{1}{2}}$. In terms of τ time, the approach of a towards a_1 will be greatly stretched out and very gradual, since as $t \rightarrow t_p$ (and a becomes small) τ approaches very large negative values. The results will be independent of the detailed behaviour of $a(t)$ near the 'singularity', but will depend on its being characterised by G, $\hbar$, and c. We regard the smooth joining of $a(t)$ to a constant a_1 as the simplest means of constructing unambiguous initial conditions incorporating the Planck dimensions, and not necessarily as a true representation of the universe 'before' the expansion began (just as 'turning on' an interaction between particles does not imply it was ever absent in nature).

As an initial condition we suppose that for large negative τ

$$\psi_k \Big|_{\tau \rightarrow -\infty} = (2ka_1{}^2)^{-1/2} \exp(-ika_1{}^2\tau)$$

so that the $A_{\mathbf{k}}$ are the usual special relativistic annihilation operators corresponding to physical particles in the initial Minkowski space ($a = a_1$). (The quantity k/a is the magnitude of the momentum.) We assume that no particles are present initially, $A_{\mathbf{k}} \, |0\rangle = 0$ for all $\mathbf{k}$, where $|0\rangle$ is the state of the system (Heisenberg picture). After $a(t)$ has settled down to the form $Ct^{\frac{1}{2}}$ there will be essentially no particle production resulting from the expansion, because, as we showed in ref. 2, or as can be directly verified from equation (2), the WKB expressions for ψ_k are exact solutions for that particular form of $a(t)$.

Fundamental approximation and solution

The key characteristics of $a(t)$ are that, viewed as a function of τ, the quantity a^4 in equation (2) gradually approaches a constant value $a_1{}^4$ for large negative τ and grows smoothly to a value large with respect to $a_1{}^4$. It is furthermore mathematically convenient (but does not affect the result, since the particle creation is negligible when a is large) to let a^4 approach a constant value $a_2{}^4$ for large positive τ, so that one has a final Minkowski space in which to unambiguously determine the results. Then, following a method which has been used in treating the reflection of radio waves and quantum mechanical reflection from a potential barrier in the regime where the WKB approximation would yield no reflection[13,14], we approximate a^4 in equation (2) by

$$a^4 \simeq a_1{}^4 + \mathrm{e}\zeta [(a_2{}^4 - a_1{}^4)(\mathrm{e}\zeta + 1) + b] (\mathrm{e}\zeta + 1)^{-2} \qquad (3)$$

where $\zeta = \tau s^{-1}$, s is a constant giving the range in τ time over which the transition from a_1 to a_2 takes place, and b is a positive constant. Our final result will be independent of b and a_2. One easily sees that for the present problem $s \simeq a_1{}^{-3} t_p$ (for example, in an expansion with $a \propto t^{\frac{1}{2}}$ starting at t_p, the τ interval for a^4

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to grow from a_1^4 to $16a_1^4$ is $\Delta\tau = a_1^{-3} t_p$, whereas the τ interval for a to grow to infinity is $2a_1^{-3} t_p$).

For large τ, we have

$$\psi_k\Big|_{\tau\to\infty} = (2ka_2^2)^{-1/2}[\alpha_k \exp(-ika_2^2\tau) + \beta_k \exp(ika_2^2\tau)]$$

where α_k and β_k are complex constants. The Wronskian condition requires that $|\alpha_k|^2 - |\beta_k|^2 = 1$. The annihilation operators $a_{\mathbf{k}}$ for particles at late times are given by $a_{\mathbf{k}} = \alpha_k A_{\mathbf{k}} + \beta_k^* A_{-\mathbf{k}}^\dagger$. The exact solution of equation (2) with a^4 given by equation (3) is discussed in refs 13 and 14, where it is shown that

$$\left|\frac{\beta_k}{\alpha_k}\right|^2 = \frac{\sin^2\pi d + \sinh^2[\pi sk(a_1^2 - a_2^2)]}{\sin^2\pi d + \sinh^2[\pi sk(a_1^2 + a_2^2)]} \tag{4}$$

where d is a real number involving the constant b. For any k significant for the highly red-shifted radiation observed in the present universe, the quantity ska_2^2 is very large compared to unity (for example, if $a_2 \simeq C t_2^{\frac{1}{2}}$ with $t_2 \simeq 10^{-28}$ s, then $ska_2^2 \gg 1$ for all k contributing to present frequencies > 1 Hz). Therefore, to an excellent approximation

$$|\beta_k/\alpha_k|^2 = \exp(-4\pi ska_1^2) \tag{5}$$

This result is independent of b and a_2 in equation (3), and depends mainly on the gradual and smooth joining to a_1 which takes place at very early times. These considerations, together with general results[15,16] about the behaviour of $|\beta_k/\alpha_k|$, suggest that an expression of the form of equation (5) holds whenever the approach of $a(\tau)$ to a_1 is sufficiently smooth. (We are not yet sure whether the exponential approach of $a^4(\tau)$ to a_1^4 as $\tau \to -\infty$ is essential; it may only be necessary that a^4 and all its derivatives be continuous and that $a_2 \gg a_1$.) The result in equation (5) is certainly independent of the (reasonable) behaviour of a when it becomes large with respect to a_1. It should hold for the actual expansion (equation (3) was introduced for mathematical convenience) by the time $|\beta_k/\alpha_k|$ ceases to change significantly (certainly no later than 10^{-20} s, and probably much closer to t_p).

Energy distribution of created particles

We have shown[1,2] that production takes place in particle pairs, and have given the probability distribution of the created particles. From equation (51) of ref. 2, we conclude that the probability of observing n pairs, where one particle is in mode **k** and the other (antiparticle) in mode -**k**, is

$$P_n(\mathbf{k}) = |\beta_k/\alpha_k|^{2n}|\alpha_k|^{-2}$$

Equation (52) of ref. 2, on the other hand, gives (in the discrete representation) the average number of particles in mode **k** in a coexpanding volume element as $\langle N_{\mathbf{k}}\rangle = |\beta_k|^2$. The correlations between particle and antiparticle of a pair are effectively destroyed by decay processes and other interactions. In this way entropy is generated by the particle creation process, and the particles (or antiparticles) alone are described by a density matrix or mixed state with the above probability distribution. Using equation (5) and $|\alpha_k|^2 - |\beta_k|^2 = 1$ in the expressions for $P_n(\mathbf{k})$ and $\langle N_k\rangle$, one readily finds that

$$P_n(\mathbf{k}) = \exp(-n\mu k)[1 - \exp(-\mu k)] \tag{6}$$

and

$$\langle N_k\rangle = [\exp(\mu k) - 1]^{-1} \tag{7}$$

where $\mu = 4\pi sa_1^2 \simeq 4\pi a_1^{-1} t_p$. This is black-body radiation with a temperature at time t given by

$$T(t) \simeq (4\pi k_B)^{-1} t_p^{-1}(a_1/a(t)) \tag{8}$$

where k_B is Boltzmann's constant, and the time dependence arises because the wavelength at time t is $(k/a(t))^{-1}$. This unexpected result permits us to associate uniform temperature and entropy with the initially created particles, even in causally unconnected regions.

This result is consistent with the Einstein equation

$$a^{-1}\mathrm{d}^2a/\mathrm{d}t^2 = -8\pi G\rho/3 \tag{9}$$

where we have used $p = \rho/3$. Putting $\rho = (\eta_a/2)\sigma T^4$, where η_a is the number of different kinds of particles produced initially (counting each state with different quantum numbers (spin and charge) separately), and $\sigma = \pi^2 k_B^4/15$ is the Stefan–Boltzmann constant, one finds from equation (9) with $a(t) = Ct^{1/2}$ and $a_1 = Ct_p^{1/2}$

$$T(t) = 2(45\pi)^{1/4}\eta_a^{-1/4}(4\pi k_B)^{-1} t_p^{-1}(a_1/a(t)) \tag{10}$$

at early times (t_p occurs here because $G = t_p^2$). This is the same as equation (8) to within a factor of $\sim 7\eta_a^{-1/4}$. This agreement is satisfactory, considering that equation (8) was obtained using rough, order of magnitude estimates, and that a dependence on η_a could appear in the expressions for s and a_1 if the early behaviour of a depends on quantum effects involving the number of fundamental fields (we are using a model with a limited number of such fields).

Consistency with observations

Finally, it is worth noting that the temperature in equation (10) (or equation (8)) can be connected to the present temperature. As the Universe expands, the original particles decay, interact, and annihilate, leaving η_b components of the present black-body radiation (counting each spin and charge state separately, and each fermion as 7/8 of a boson in forming the entropy below). Assuming that the entropy

$$S = \eta(t)(2/3)\sigma T^3(t) a^3(t)$$

in a comoving volume, $a^3(t)$, is conserved, one finds[17-19]

$$T_b \simeq (\eta(t)/\eta_b)^{1/3}\, T(t)\,(a(t)/a_b) \tag{11}$$

where subscript b refers to the present time, and $\eta(t)$ is the number of elementary constituents at time t. Assuming that the factors involving η are ~1, since powers like 1/3 or 1/4 are involved, one finds from equations (10) and (11) that (in c.g.s. units)

$$T_b \simeq 7(4\pi k_B)^{-1}\,\hbar t_p^{-1}\,(a_1/a_b) \tag{12}$$

Denoting the earliest time at which the universe becomes matter-dominated by t_e (so that $a \propto t^{2/3}$ for $t > t_e$), one finds $a_1/a_b \simeq (3/2)^{2/3}(t_p/t_H)^{1/2}(t_e/t_H)^{1/6}$, where $t_H = a(\mathrm{d}a/\mathrm{d}t)_b^{-1} = 3t_b/2$ is the Hubble time[20], $t_H = 5.8 \times 10^{17}$ s. This gives

$$T_b \simeq (t_e/t_H)^{1/6}\,(30\ \mathrm{K}) \tag{13}$$

which equals 3 K when $t_e \simeq 2 \times 10^4$ yr, but is within a factor of 10 of 3 K for all possible values of t_e > 8 d. To determine t_e in our model one would have to predict the baryon number density (or entropy per baryon).

Any theory consistent with Einstein's equations and leading to black-body radiation before t_e will yield equation (12) and (13), since equation (10) (with η_a replaced by $\eta(t)$) follows from the Einstein equation (9) at a time t shortly before t_e. The particle creation process occurring near t_p leads naturally, however, to the interpretation of T_b in equation (12) as the redshifted temperature of the particles created with a temperature of the order of the Planck temperature ($k_B^{-1}\hbar t_p \simeq 10^{32}$ K). In that sense, a model of the present kind (or possibly starting with an anisotropic expansion) seems to point towards a plausible explanation of the origin of the density and entropy of the black-body radiation. Our model is perhaps the simplest of those involving particle creation near the Planck time.

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Abundance and membrane association of elongation factor Tu in *E. coli*

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Elongation factor Tu is present in a fourfold excess over the amount seemingly involved in protein synthesis. It is identical to a major protein which we have found associated with the plasma membrane. The possibility of a specific role in that location is considered.

In a recent study of envelope-associated proteins, we have described the properties of a homogeneous polypeptide of which there are 70,000 molecules per cell (5% of the total cellular protein)[1]. Several observations, particularly its release from the cell by osmotic shock but not by the conversion of cells to spheroplasts, suggested that this polypeptide is probably associated with the inner face of the plasma membrane *in vivo*. We could not identify the function of this protein with a molecular weight of 44,000. We now report that it is identical with elongation factor Tu (EF Tu) which through cyclic association and dissociation with another factor, EF Ts (ref. 2), is an essential component in protein synthesis (for review, see ref. 3). We demonstrate that EF Tu is present in substantial excess over EF Ts, and therefore over ribosomes[4]. This abundance and the suggested association with the membrane led us to ask what function EF Tu could exert in that location.

EF Tu and the protein released from the cell by osmotic shock are identical

The relationship between the protein we have described[1] and EF Tu is shown by the following observations. Electrophoresis in sodium dodecyl sulphate (SDS) of authentic EF Tu, purified according to Miller and Weissbach[5], and of the major protein released by osmotic shock yielded identical mobilities for the two proteins (not shown). The amino acid compositions of the two proteins are very similar[1,5], and well within the variations usually observed between laboratories. The amino terminal ends of both proteins are blocked (ref. 1 and Wade *et al.*, unpublished, cited in ref. 6). We have confirmed this for authentic EF Tu by the method of Weiner *et al.*[7]. No aminoacyl residue modified in its α-amino group was found after dansylation and hydrolysis of the undegraded polypeptide and after each of three successive cycles of Edman degradation. The spectra of the membrane-associated protein before and after extensive dialysis[1] are identical with the corresponding spectra of EF Tu (ref. 5). The functional identity of the released protein with EF Tu was tested by measuring both GDP binding[5] and stimulation of the *in vitro* polymerisation of phenylalanine[8] in osmotic shock supernatants. The results (Table 1) show that the activities characteristic for EF Tu vary according to the amount of the major protein released into the shock fluid. To determine whether the two proteins are in fact the same, we prepared tryptic peptide maps of the purified membrane-associated protein and of EF Tu. The fingerprints obtained were very similar (Fig. 1*a* and *b*). Double-diffusion tests of antibodies specific to one

Table 1 Correlation between release of EF Tu and of the major membrane-associated protein released from *E. coli* by osmotic shock*

Shock medium	Phe polymerisation† (pmol polymerised ml^{-1})	GDP binding‡ (nmol bound ml^{-1})	Amount of membrane-associated polypeptide§ (% total cell protein)
Water			
Supernatant¶	74 (80%)‖	4.0 (51%)‖	3.6 (74%)‖
Pellet¶	18 (20%)	3.8 (49%)	1.3 (26%)
0.5 mM $MgCl_2$			
Supernatant¶	21 (25%)	0.76 (9%)	0.4 (8%)
Pellet¶	62 (75%)	7.4 (91%)	4.4 (92%)

*Cells (*E. coli* K12, derivative BHB 960) were grown to a density of 6×10^{8} ml^{-1} and osmotically shocked using the procedure of Nossal and Heppel[9] with H_2O or 0.5 mM $MgCl_2$ as shock medium. Equal aliquots of the supernatants and pellets, taken up in the original volume and sonicated, were assayed as indicated below. Total activities were determined for all experiments by assaying sonicated, but unfractionated, cells. These were always within 10% of the sum of osmotic shock supernatants and pellets. Cell lysis was monitored by measuring β-galactosidase activity[10] in supernatants and pellets, and was always $\leqslant$20% with water and $\leqslant$10% with $MgCl_2$.

†Phenylalanine polymerisation[8] using ^{14}C-phenylalanine tRNA and washed ribosomes as described previously[11] was measured for 5-min periods at 30 °C. EF Ts (purified as described in Fig. 2) and EFG free of EF Tu activity (an S-100 of *E. coli* BE was treated at 55 °C for 15 min, ref. 12) were added in excess. Purified EF Tu polymerised 0.80 nmol Phe per 5 min per mg protein in these conditions, close to the value of 0.88 calculated from ref. 8.

‡Binding was measured with 5 μM ^{14}C-GDP (Amersham) by filtering the reaction mixture after 15 min at 25 °C through nitrocellulose filters (Sartorius, 0.45-μM pore size).

§Estimated by quantification of the peaks representing the membrane-associated polypeptide in densitometric scans of stained polyacrylamide slab gels, as described previously[13].

¶Protein concentrations were: 0.6 and 8.5 mg ml^{-1} in the shock fluid and the residue, respectively, in the presence of magnesium ions, and 2.5 and 7.5 mg ml^{-1} in its absence.

‖Figures in parentheses indicate the percentage of the total activity or of the total membrane-associated protein, respectively.

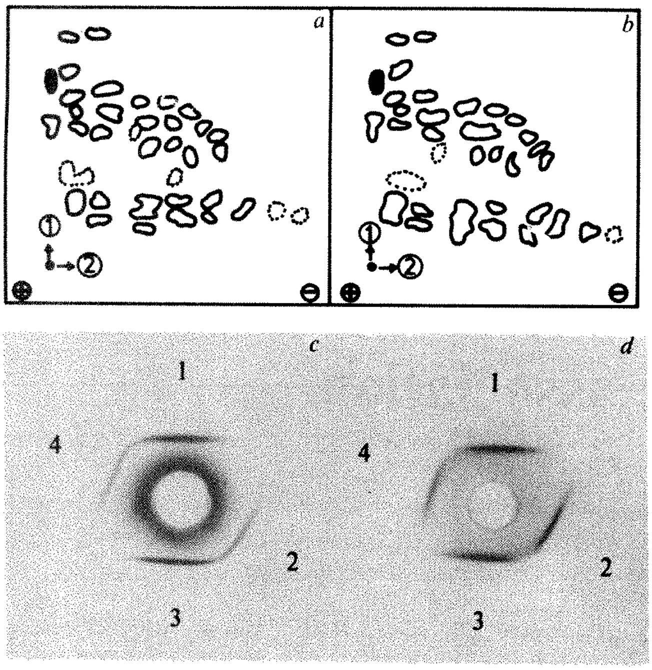

Fig. 1 Comparison of osmotically released polypeptide and EF Tu. The two peptide maps represent the patterns after ninhydrin staining (*a*) and an autoradiogram (*b*) of the same chromatogram. *E. coli* BE cells were grown as described previously[13] and labelled with a ^{14}C-amino acid mixture at a cell density of 10^8 ml^{-1}. Cells were collected at 6×10^8 ml^{-1}, washed and subjected to osmotic shock. Concentrated shock fluid was subjected to gel electrophoresis in SDS. The major band (44,000) was eluted, and the concentration of detergent lowered by dialysis overnight at low ionic strength. A tenfold excess of pure, unlabelled EF Tu (ref. 5) was then added. The mixture was digested with TPCK-treated trypsin and subjected first to chromatography (dimension 1) and then electrophoresis (dimension 2) on silica gel thin layer plates (Kodak 6061) with solvent systems as described previously[14]. The minor differences of the maps, if they are not artefactual, may be due to the fact that there are two genes coding for EF Tu (ref. 15), and that the two gene products have been enriched to different extents in the preparation procedures for EF Tu and the protein released by osmotic shock. From the number of spots (34) and the sum of arginyl and lysyl residues in the two proteins[1,5], the possibility of a mixture of two non-identical proteins can be excluded. The Ouchterlony double-diffusion test shows the reaction of antibodies specific for the osmotically released protein with EF Tu (wells 1 and 3) and an osmotic shock supernatant (wells 2 and 4), (*c*); and the reaction of antibodies specific for EF Tu with the same samples in the outer wells (*d*).

protein yielded precipitin lines continuous with that of the other (Fig. 1*c* and *d*).

Excess of EF Tu over EF Ts in the cell

It has been reported that EFs are synthesised in stoichiometric amounts with ribosomes, and that their quantities in the cell vary with the growth rate of bacteria[4]. In the case of EF T, this observation should be restricted to EF Ts, since the antibodies, used for the quantifying of the factors, were specific for EF Ts, and did not interact with EF Tu (ref. 16). In view of the fact that there are 70,000 molecules of EF Tu per cell[1], and that we found little variation in the different growth conditions used, we re-determined the proportions of EF Tu and EF Ts in the cell.

First, we determined the amounts of the two factors in the three fractions from an ammonium sulphate precipitate, which represented an intermediate step in the purification procedure[17]. Table 2 shows that almost two-thirds of the total EF Tu was contained in fraction C. Activity determined in that fraction by the phenylalanine polymerisation assay, however, is considerably less[17], an observation explained by the virtual absence of EF Ts in that fraction. This result was confirmed by gel filtration chromatographic analyses of extracts A, B and C (Fig. 2). EF Tu is therefore presumably underestimated when an activity assay designed to detect the function of the complexed factor (EF T) is used, but is revealed by gel electrophoretic analyses of the various fractions.

From the data in Table 2 and Fig. 2, it can be estimated that approximately 80% of total EF Tu occurs in the non-complexed form. The ratio of EF Tu to EF Ts would be 5 : 1. To confirm this result, we used an independent method. The estimation of the amount of protein in the bands on SDS gels with mobilities corresponding to EF Tu and EF Ts (Fig. 3) yielded a ratio of 3.6±0.4 (Table 3). Although we have already determined that the band corresponding to EF Tu consists of at least 90% of this factor[1], we do not know whether other proteins comigrate in the position of EF Ts; a value of 3.2 is therefore a minimal estimate. It is reasonable to conclude, therefore, that the two factors occur in a ratio of approximately 4 : 1, independent of the growth conditions used.

Table 2 Quantification of EF Tu from stained gels and EF Ts by activity assays in ammonium sulphate fractions*

Extract (% $(NH_4)_2SO_4$)	EF Tu† (% total)	EF Ts‡ (% total)
A (27.8% eluate)	7.8	25
B (24% eluate)	28.2	75
C (19.6% eluate)	64.0§	None detected

*A crude extract of 100 g *E. coli* BE was carried through the ammonium sulphate fractionation step[17] in the purification of EF T and EF G. No EF Tu or EF T was found in the residue after the 19.6% extraction.

†Estimated from densitometric scans of stained SDS–polyacrylamide gels[13] of the three fractions.

‡Measured by exchange[18] of ^{14}C-GDP with purified EF Tu–GDP.

§Our finding of large quantities of non-complexed EF Tu is in agreement with results of Arai *et al.*[19], who purified EF T, EF Tu and EF Ts by ion exchange chromatography in the presence or absence of GDP. They also found a large fraction of free EF Tu in cell extracts.

Also presented in Table 3 are the growth rates obtained for *Escherichia coli* BE cells in different media and the corresponding values for the proportion of total cell protein represented by EF Tu. Cells growing very slowly synthesised about half the amount of EF Tu as those grown in richer media. Since the ratio of the two factors remains the same (3.4), there is approximately half as much EF Ts in cells grown on the poorer medium. Although with the strain used in this study, minimal media containing glucose still yielded short generation times (Table 3), our results are consistent with the previously published variation of the level of EF T with growth rate[4]. The synthesis of EF Tu thus seems to be coordinated with that of ribosomes and EF Ts, but at a much higher level.

Possible functions for EF Tu at the membrane

Since EF T occurs in an approximately equimolar ratio to ribosomes[4], and this complex is known to contain EF Tu and EF Ts in stoichiometric amounts, 20–30% of the total complement of EF Tu seems to be involved in protein synthesis at any given time. The simplest explanation for the 'excess' of EF would be that the binding of aminoacyl-transfer RNA to the acceptor site in the ribosomes is inefficient, and that a large excess of the protein responsible for this process is required for effective protein synthesis. Alternatively, or in addition, EF Tu may be responsible for other functions in the cell. It is well known that when *E. coli* is infected with RNA phages such as Qβ, this protein becomes a subunit in the enzyme responsible for RNA replication[23]. It may further exert a control function in transcription, in conjunction with the effector

Table 3 Stoichiometry of EF Tu and EF Ts

Method	Medium	Generation time (h)	EF Tu (% total protein)*	EF Tu/EF Ts (mol per mol)†
Quantification by Sephadex G-100 chromatography of ammonium sulphate fractions	M9 (minimal)	0.87	—	5.1‡
Stain quantification from gels	M9 (minimal)§	0.87	5.1	3.3
	M9+1% casamino acids mixture (Difco)	0.48	4.6	4.0
	Tryptone broth (Difco)	0.48	4.5	3.8
	Acetate (1%)	4.5	2.7	3.4
Determination of radioactivity¶ eluted from gels	M9 (minimal)	0.87	—	3.5

*Calculated from densitometric scans of SDS gels (see Fig. 3).

†Bands corresponding to EF Tu and EF Ts were cut from stained gels. The stain was eluted with dimethylsulphoxide and the A_{600} of eluates measured (for details, see ref. 22). Each value is the average of at least four determinations.

‡Calculated from data given in Table 2 and the integrated peaks in Fig. 2.

§Cells were grown to densities of 6×10^8 ml^{-1} in the media indicated, collected, dissolved directly in SDS, and subjected to gel electrophoresis in the detergent.

¶Cultures in M9 medium were labelled at cell densities of 10^8 ml^{-1} with 0.25 μCi-ml^{-1} U-^{14}C-amino acid mixture (Amersham). At densities of 6×10^8 ml^{-1}, they were collected and electrophoresed as described above. Bands were dissolved in 30% H_2O_2 at 60 °C overnight and counted as described previously[22]. The value given is the average of four determinations.

guanosine tetraphosphate[24,25]. It is unclear, however, whether these functions could account for the large amount of EF Tu, and for the high ratio of EF Tu to Ts.

We have shown[1] that 50–80% of EF Tu is released from the cell by osmotic shock (data corrected for cell lysis). We have suggested that the released fraction is associated with the inner face of the plasma membrane, suggesting that a specific function is carried out in that location. Membrane association of many processes involved in macromolecular synthesis in bacteria has been suggested[26], and some of these, in turn, may be related to EF Tu (see above).

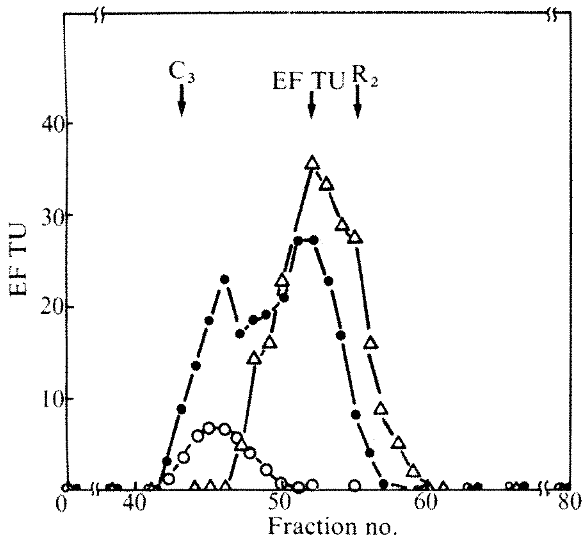

Fig. 2 Gel filtration chromatographic analyses of the three extracts from an ammonium sulphate fractionation (see Table 2). Eluates A, B and C were dialysed into 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol and 0.1 M KCl, and chromatographed on a calibrated Sephadex G-100 column in the same buffer. All fractions containing material absorbing at 280 nm were subjected to electrophoretic analysis in SDS. The gels were stained with Coomassie blue. From the patterns obtained, the amount of EF Tu was quantified by integration of the peaks obtained by scanning densitometry (for details, see ref. 13). Units on the ordinate are arbitrary. ○, Extract A; ●, extract B; △, extract C. C_3 and R_2 indicate the catalytic and regulatory subunits of aspartate transcarbamylase, with molecular weights of 100,000 and 34,000, respectively[20]. All EF Tu in extract A and 40% of it in extract B chromatographed with a distribution coefficient (K_d) corresponding to a molecular weight of 80,000. The remaining 60% in extract B and all in extract C had a K_d identical to that of purified EF Tu (arrow). The composition of the complexed form of EF Tu (80,000) was determined by rechromatography of the pooled fractions (43–46) in the presence of 1 mM GDP in the column buffer. In these conditions, EF Tu eluted quantitatively at the position indicated. The only other polypeptide to shift position in this second dimension was one with a mass of 32,000. The protein is present in approximately stoichiometric amounts with EF Tu. Its identity to EF Ts was confirmed by separating the two proteins which change their elution position in the presence of GDP using ion exchange chromatography[5]. Determination[19] of EF Ts activity yielded 2.7×10^5 U mg^{-1} in the peak containing the 32,000 polypeptide. We have used this two-dimensional procedure to purify EF Ts.

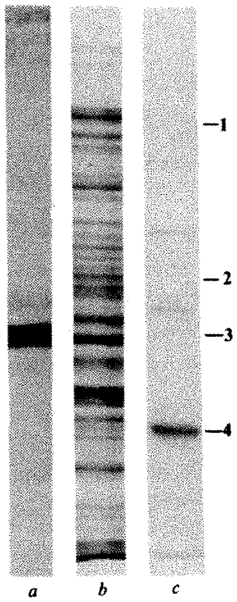

Fig. 3 Gel electrophoretic patterns in SDS of pure EF Tu (*a*), whole cells directly dissolved in sample buffer[21] (*b*) and EF Ts, purified according to the two-dimensional procedure given in Fig. 2 (*c*). Scanning densitometry indicated that the main band of gel *c* constitutes 80% of the total protein applied. Determination of EF Ts activity in the GDP-exchange reaction[18] yielded a value 20% smaller than that found for the pure factor[18]. It can therefore be concluded that the major band is pure EF Ts. The growth conditions and the method for estimating the intensities of the bands corresponding to EF Tu and EF Ts in gel *b* are described in Table 3. Molecular weight standards indicated were: 1, phosphorylase *a* (95,000); 2, catalase (58,000); 3, ovalbumin (45,000); 4, C chain of aspartate transcarbamylase (33,000).

The protein may, of course, also be functional in the synthesis of proteins on membrane-bound ribosomes[27]. The evidence for a compartmentalisation of ribosomes in bacterial cells, however, is not fully convincing; according to recent estimates[28], it would account for only one-third of the ribosomes and therefore for less than 10% of EF Tu.

One observation may be of value in determining whether EF Tu provides a link between several cellular processes in the membrane, or whether it may have other functions there. Morrissey *et al.*[29] have reported that the ribosomal proteins L7/12, known to interact with EF Tu on ribosomes (see ref. 30 for references), also occur in the non-ribosomal fraction of the cell in amounts similar[29] to those of 'excess' EF Tu. It would be interesting, therefore, to determine whether these proteins also interact with EF Tu at other cell sites, and if so, whether the function of the complex may be more amenable to analysis than that of either component alone.

Yet another possibility is suggested by our observation that the membrane-associated fraction of EF Tu is partially precipitated by calcium, and quantitatively by vinblastine ions. We have considered the possibility that these and related observations may indicate a structural role for this protein at the bacterial membrane[1]. Finally, Jaskunas *et al.*[15] have reported that at least two genes code for EF Tu. Although the products of these genes seem to be very similar[15], this observation opens up the possibility of a genetic approach to investigating the functions of EF Tu in the cell.

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Note added in proof: Furano[31] has also reported a high excess of EF Tu over ribosomes. According to his interpretation, the excess of the elongation factor allows binding of the entire aminoacyl-tRNA pool in the cell.

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Production of yeast alcohol dehydrogenase isoenzymes by selection

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Mutants of yeast alcohol dehydrogenase have been produced that protect the cell against the poisonous aldehyde acrolein by increasing the NADH–NAD ratio. The altered properties include changes both in binding constants and in cooperativity. Such mutants may be useful in exploring the nature of adaptation at the molecular level.

WORK in the field of molecular 'evolution' in the laboratory has so far been directed towards the selection of mutant enzymes with a high affinity towards unusual substrates[1–3]. I report here on a specific selective system that produces mutants of yeast alcohol dehydrogenase (ADH, EC 1.1.1.1) in which other functions have been altered. The kinetic alterations are sufficiently marked to justify the view that these mutant gene products are new isoenzymes. There is at this locus a potential for great diversity in adaptational response, which may cast some light on the fine details of the evolutionary process.

The selection procedure has been set forth in detail elsewhere[4], and relies on two observations. The first is that petite yeasts (those unable to respire aerobically) synthesise only one of the two isoenzymes found in the cytoplasm. This form, ADH-I (ref. 4), is normally constitutive under laboratory conditions, has a high K_m (Michaelis–Menten constant) for ethanol, and seems to be chiefly responsible for the production of ethanol from acetaldehyde in cells grown anaerobically[5]. The other cytoplasmic isoenzyme, ADH-II, is coded by an unlinked locus[6], has a much lower K_m for ethanol, and has a relative mobility at *p*H 8.6, with ADH-I taken as 100%, of 110%. ADH-II is found only in aerobically grown grande yeast. A primary function of ADH-II is to convert ethanol accumulated during anaerobic growth to acetaldehyde, with the concomitant reduction of NAD. ADH-I can also accomplish this task, though presumably less efficiently; grande mutants (those able to respire aerobically) free of ADH-II have been produced in several laboratories[4,7] and grow satisfactorily on ethanol as the sole carbon source.

The second observation on which the selection scheme is based is that grande cells lacking cytoplasmic ADH activity cannot be induced to become petite. They die instead, indicating

Table 1 Concentrations of NADH and NAD in crude extracts of wild-type and mutant cells

Strain	μg NADH per mg protein	μg NAD per mg protein	NADH–NAD ratio
XW517-2D (ADH-I)	0.035	0.568	0.062
XW520-2A (ADH-II)	0.152	0.913	0.166
XW517-S-AA-1	0.296	0.781	0.379
XW517-S-AA-5	0.196	1.038	0.188

Protein was determined by the method of Lowry *et al.*[15]. Cells were grown in YEPD+2% EtOH. Average of two determinations.

that ADH activity is essential for the survival of petite cells. Reinforcing this conclusion, it has proved impossible to select petite strains to yield cells lacking ADH activity. It seems likely that glycolysis cannot proceed without the presence of ADH to catalyse the production of ethanol as a final step, although grande cells lacking ADH activity can survive by means of oxidative phosphorylation alone.

Cells lacking ADH activity and ADHs with altered kinetics were selected by an elaboration of the scheme originally used by Megnet[8]. Allyl alcohol, CH_2CHCH_2OH, is readily oxidised by yeast ADH to acrolein (acrylaldehyde). Acrolein is highly toxic to yeast cells, although the mechanism of its toxicity is in doubt[9]. Selection on a specific sequence of media with increasing allyl alcohol concentrations resulted in the production of grande yeast strains lacking both cytoplasmic and mitochondrial ADHs (ref. 3). These strains could survive in allyl alcohol concentrations up to 1.6 M, compared with 1 mM for the wild type, which was a strong indication that allyl alcohol by itself was harmless to the cell. These were the strains that could not be converted to petite, demonstrating that petites lacking ADH activity could not survive.

Table 2 Acetaldehyde and ethanol in medium of cells grown anaerobically to log phase

Strain	Acetaldehyde (mM)	Ethanol (M)
XW517-2D (ADH-I)	1.2	0.096
S-AA-1	4.84	0.05
S-AA-5	1.2	0.12

Concentrations were measured enzymatically with commercial ADH (Sigma). Cells were grown in a New Brunswick Bio-Flo chemostat, model C-20, modified by the addition of a closed-circuit pump passing medium and cells from the instrument through a photocell monitor connected by a feedback loop to the chemostat's medium pump. The concentration of cells in the chemostat chamber could therefore be kept constant and at log phase. All strains were grown to a level of 3×10^7 cells ml^{-1}, without aeration, since introduction of air rapidly removes acetaldehyde from the medium by evaporation. Thus, the pattern of excretion of the strain producing ADH-II could not be checked accurately since in anaerobic conditions it also produced only ADH-I.

The rationale for producing mutant enzymes with altered kinetics was therefore straightforward. Selection was carried out on petite strains grown on complete medium with increasing amounts of allyl alcohol. The assumption was that petite strains could not simply escape by mutating to ADH$^-$, since they required ADH activity. Only those cells containing an enzyme that could somehow discriminate between the normal substrate, ethanol, and the potentially dangerous one, allyl alcohol, would survive. It was assumed initially that the mutant enzymes thus obtained would show marked kinetic differences between the two substrates, but this was not the case and in retrospect it can be seen to be unlikely because of the similarity between the two molecules. Instead, the mutant enzymes changed the intracellular environment so as to shift the equilibrium between allyl alcohol and acrolein in the direction of the harmless alcohol. This was done by shifting the NADH–NAD ratio.

Selection and physiological effect of mutant enzymes

The mutants were selected from a wild-type cytoplasmic petite, XW517-2D, *a* mating type, by plating on YEPD (1% yeast extract, 2% Bacto-peptone, 2% dextrose, all w/v, all from Difco) to which 2% ethanol (v/v) and allyl alcohol had been added. Spontaneous mutants were selected that grew well at 20, 40 and 60 mM allyl alcohol, cloned at each stage and plated at the next highest concentration. Vigorous clones from the highest concentration of allyl alcohol were used.

Two independently derived mutants were chosen to be investigated thoroughly, XW517-S-AA-1 and XW517-S-AA-5. Each migrated electrophoretically more slowly than the ADH-I from XW517-2D at *p*H 8.6, 93% and 85% respectively. Both were shown to be allelic to ADH-I and not to ADH-II by the appropriate crosses. When tetrads from crosses of the mutant to the wild type were made petite, resistance to 60 mM allyl alcohol segregated 2:2 in the tetrads and was invariably found to be associated with the mutant enzyme.

A further useful spontaneous mutation occurred in one of the segregants from a cross involving S-AA-1. This strain, which was thought to be petite, was found to produce ADH-II, and grew very slowly on glycerol as the sole carbon source. The strain had been converted to petite using ethidium bromide[10], but the conversion was incomplete. This enabled us to investigate the physiological properties of ADH-II on a genetic background that was almost petite, and to compare them with the properties of the mutant enzymes and of ADH-I. This strain was designated XW520-2A.

Kinetic studies of the mutant enzymes showed that there was no pronounced change in their affinity for allyl alcohol. Indeed, the kinetic changes were slight and did not involve a large increase in K_m for this substrate. This was puzzling until we realised that neither allyl alcohol nor acrolein is likely to be metabolised further by the cell. In vivo therefore, they should eventually reach some approximation of their apparent thermodynamic equilibrium

$$K'_{eq} = \frac{[\text{Acrolein}]\,[\text{NADH}]}{[\text{Allyl alcohol}]\,[\text{NAD}]}$$

complicated by the possibility that substrate and product may be acted on differentially by active transport systems.

This equilibrium could not be determined precisely because of side reactions entered into by the acrolein, but it approximates that for ethanol and acetaldehyde (1.15×10^{-4} at *p*H 7.0 (ref. 11)). Thus, an increase in the NADH–NAD ratio in the cell will produce a corresponding decrease in the acrolein–allyl alcohol ratio. Since the last compound is harmless, this should protect the cell. I therefore investigated changes in the ratio and changes in the physiology of the mutant cells.

Oxidised and reduced NAD were measured in whole-cell preparations by the Everse *et al.*[12] modification of the method of Greengard *et al.*[13] (Table 1). All three mutants, including the near-petite that produces primarily ADH-II, showed a pronounced increase of reduced relative to oxidised NAD.

Such a shift should be associated with a shift in the relative amounts of ethanol and acetaldehyde excreted by the mutant cells. The amounts of acetaldehyde and ethanol in the medium, measured enzymatically, are shown in Table 2. The two mutants show different patterns, with S-AA-5 producing more ethanol and S-AA-1 producing more acetaldehyde than the wild type. As we shall show, this was consistent with the kinetic changes.

If a shift in the NADH–NAD balance in the cell is responsible for resistance, growing the cells in conditions designed to restore the original balance should abolish the resistance, and that is what happened. It had been observed that acetaldehyde is lost rapidly from aerated but not from non-aerated cultures. The reaction should therefore be driven in the direction of ethanol in a closed system, but not in one in which the acetaldehyde is given a chance to escape. Further, addition of ethanol, even in anaerobic conditions, should drive the reaction back towards acetaldehyde, restoring the resistance. S-AA-1 and the ADH-II mutant showed the expected pattern, growing well under aeration in the presence of 20 mM allyl alcohol, but not without aeration. The resistance was readily restored in anaerobic cultures by the addition of ethanol. S-AA-5, however, showed some growth at 20 mM allyl alcohol without aeration. Ethanol also increased the resistance of this mutant. It thus seemed likely that the resistance of these mutants could be directly traceable to an enzyme-mediated shift in the proportions of reduced to oxidised pyridine nucleotides. Detailed kinetic studies bore this out, and cast some light both on the mechanisms of the wild-type isoenzymes and on how these mechanisms have been altered in the mutants.

Kinetics of wild-type and mutant enzymes

Table 3 gives the kinetic measurements of the ethanol–acetaldehyde reaction for the two wild-type isoenzymes and the two mutants. Allyl alcohol kinetics were also obtained, and showed differences analogous to those for ethanol, but it has so far proved impossible to obtain consistent acrolein kinetics because of side reactions. A number of kinetic parameters were obtained, as detailed in the legend to Table 3. K_A and K_B are the K_ms for substrate at infinite cofactor concentration and cofactor at infinite substrate concentration respectively; K_{AB} is the K_m for substrate and cofactor.

The kinetic data can be considered on two levels: the differences in K_m and V_{max}, and the differences in cooperativity. Both are approximately consistent with the physiological data and with the working hypothesis concerning the nature of allyl alcohol resistance.

ADH-I and ADH-II do not differ greatly in the V_f/V_b ratio, but show markedly different K_ms. ADH-II exhibits an extreme reduction in both K_{ABf} and K_{ABb}, which is what one would expect with an isoenzyme primarily concerned with oxidation of ethanol to acetaldehyde. In aerobic conditions, acetaldehyde must swiftly be converted to acetyl CoA, so the very low K_{ABb} does not normally prevent the reaction from proceeding towards acetaldehyde. The almost petite strain XW-520-2A allows accumulation of acetaldehyde and NADH, however, which explains why this strain is resistant to allyl alcohol.

S-AA-1, which excretes four times the usual amount of acetaldehyde and loses its resistance to allyl alcohol in anaerobic conditions, shows a twofold reduction in the velocity of the back reaction coupled with a twofold reduction in the K_{ABb}. S-AA-5, which excretes more ethanol than the wild type, shows a fivefold reduction in both K_{ABf} and K_{ABb}. Although the kinetic alterations in S-AA-1 might themselves be enough to explain the shifts observed in the NADH–NAD ratio and in the pattern of excretion, they do not seem to help in the case of S-AA-5. It is also possible that some of the NADH–NAD shift has been caused by enzyme-mediated *p*H changes, and this is under investigation.

It is possible to obtain a rough estimate of the size and direction of cooperativity in these enzymes by calculating the ratios of K_{AB} to the product of K_A and K_B. The approximate ratios are summarised in Table 4. A ratio greater than one indicates positive cooperativity, with a greater ease of catalysis as the concentration of substrate or cofactor rises. The primary plots show an increasing K_m with decreasing substrate and decreasing cofactor concentration. A ratio less than one, on the other hand, indicates negative cooperativity[14], marked by a decreasing K_m with decreasing substrate or cofactor concentration. The direction of cooperativity observed in the two wild-type isoenzymes, ADH-I and ADH-II, helps to explain their preferred direction. ADH-I shows positive cooperativity for the forward reaction, and thus at low substrate and cofactor concentrations will exhibit a reduced velocity in this direction. ADH-II shows negative cooperativity for this same reaction, and this, coupled with the greatly reduced K_m, must considerably enhance its forward reaction rate at low concentrations.

The cooperativities of the two mutants have been altered in

Table 3 Kinetic constants for the four enzymes

Enzyme	Activity*	V_f/V_b	K_{ABf} (M²)	K_{ABb} (M²)	K'_{eq} (calculated) × 10³
ADH-I	352	0.14	10^{-5}	5.0×10^{-7}	6.8
ADH-II	358	0.215	1.4×10^{-7}	1.1×10^{-9}	9.4
S-AA-1	296	0.35	10^{-5}	2.4×10^{-7}	7.2
S-AA-5	79	0.122	1.8×10^{-6}	1.1×10^{-7}	7.2

Enzyme	K_{EtOH} (M)	K_{NAD} (M)	$K_{\mathrm{EtOH}}\cdot K_{\mathrm{NAD}}$ (M²)	K_{Acet} (M)	K_{NADH} (M)	$K_{\mathrm{Acet}}\cdot K_{\mathrm{NADH}}$ (M²)
ADH-I	2.4×10^{-2}	2.4×10^{-4}	5.8×10^{-6}	3.4×10^{-3}	1.4×10^{-4}	4.7×10^{-7}
ADH-II	2.7×10^{-3}	1.4×10^{-4}	3.8×10^{-7}	4.5×10^{-5}	2.8×10^{-5}	1.3×10^{-9}
S-AA-1	4.2×10^{-2}	3.1×10^{-4}	1.3×10^{-5}	1.1×10^{-3}	1.2×10^{-4}	1.3×10^{-7}
S-AA-5	2.4×10^{-2}	6.0×10^{-5}	1.4×10^{-6}	2.2×10^{-3}	6.0×10^{-5}	1.3×10^{-7}

* Units mg^{-1} purified enzyme, where one unit reduced 1 μM NAD min^{-1} at 25 °C (*p*H 8.8) in the presence of 0.3 M EtOH (ref. 16).

Enzymes were purified by treatment with protamine sulphate, a heat step and ammonium sulphate fractionation. The active fraction was redissolved in 0.01 M KPO_4 (*p*H 7.0) and passed through an affinity column of DEAE–Sephadex conjugated with 8-(6-aminohexyl)-amino-NAD (ref. 17). Elution was with a pulse of NAD, and the enzyme was further purified by passage through a small DE11 column. Purity was checked by thin-layer sodium dodecyl sulphate slab acrylamide electrophoresis. Yield was typically about 80% of the activity in the crude extract All kinetic measurements were made at *p*H 8.8 and 23 °C. NAD and NADH, purchased from Sigma, were dissolved in 0.005 M phosphate buffer, *p*H 6.0 and 8.5 respectively, put on a 2×35-cm DE11 column and eluted with a 0.005–0.2-M gradient of the same buffer. The NAD purification was essentially the same as that of Dalziel[18]; the NADH purification yielded cofactor of excellent purity with an absorbance ratio (A_{260}/A_{340}) of only 2.25. In both purifications, other peaks were observed in the gradient Purified cofactors were kept in the refrigerator in tightly-stoppered bottles and used within a week. No changes in their absorption properties were noted. Actaldehyde was distilled before use and stored at −70 °C. The enzyme was dialysed extensively before use to remove any traces of mercaptoethanol. Kinetics were measured by monitoring the change in absorbance at 240 nm recorded at the maximum sensitivity (0.1 absorbance unit full-scale) of a Gilford 2400S spectrophotometer. Velocity readings were taken for eight different substrate and six different cofactor concentrations, each 2/3 the concentration of the preceding one. The reaction was measured in 3-ml cuvettes, using 0.032 M pyrophosphate buffer. It was found that zinc greatly enhanced the velocity of the back reaction (acetaldehyde to ethanol), while not affecting the forward reaction All back reaction velocities were therefore obtained in the presence of 5×10^{-5} M $ZnCl_2$. Measurements were made in duplicate. It soon became obvious that the enzymes did not exhibit simple Lineweaver–Burk kinetics, though a Lineweaver–Burk assumption was used in the analysis. Kinetic constants were obtained by the method of Florini and Vestling[19]. A program written in BASIC for the Hewlett–Packard 9830 mini-computer was used to fit the eight constant-substrate lines and six constant-cofactor lines by least squares to Lineweaver–Burk plots. Then, secondary plots were derived from the inverses of the Vs calculated from these primary plots to obtain K_A and K_B, the Michaelis–Menten constants for the substrate at infinite cofactor concentration and cofactor at infinite substrate concentration respectively. V_{max} for the reaction was also obtained from these secondary plots. Theoretically, it should be possible to derive K_{AB} (in mol²), the Michaelis–Menten constant for substrate and cofactor, since the intersection of the lines in the primary plot has the coordinates $-K_B/K_{AB}$, $(K_{AB}-K_AK_B)/K_{AB}V_{max}$. A K_{AB} different from the product of K_A and K_B indicates the presence of interactions. The lines on the primary plot never intersected at a single point, however, showing instead a complex pattern of several intersection points The computer was instructed to print out values of K_{AB} derived from the x and y coordinates of each of the 15 constant-cofactor line intersections and the 28 constant-substrate line intersections. The two values of each pair rarely agreed with each other, but when they did, each pair that was in agreement was also found to be close to the same value. This value was taken to be K_{AB}, and could be compared with the product of the values of K_A and K_B derived from the secondary plot This procedure might seem arbitrary, since so much information was discarded, but fortunately a further check existed. Since kinetics for both the forward and the back reaction were available, it was possible to substitute them into the Haldane equation[20] $K'_{eq} = (V_fK_{ABb})/(V_bK_{ABf})$ where V_f and V_b are the maximum velocities of the forward and back reactions respectively, and K_{ABf} and K_{ABb} the Michaelis–Menten constants for the forward and back reactions. As can be seen from the table, the K_{AB}s fit the K'_{eq} derived from equilibrium measurements[11] quite well, the derived value being 7.25×10^{-3} at *p*H 8.8.

different ways. In S-AA-1, positive cooperativity has been introduced into the back reaction, which has the effect of decreasing its relative rate at low concentrations. The net result, when coupled with the K_m and V_{max} changes, will be less ethanol and more acetaldehyde excreted. In S-AA-5, the positive cooperativity of the forward reaction has been reduced or lost, which should make the reaction proceed more readily at low concentrations, yet this mutant is observed to excrete relatively more ethanol than the wild type. This shift in excretion may somehow be linked to the reduced K_ms, but the link between the kinetic changes and the NADH–NAD shift is not as clear as in S-AA-1.

It seems likely from the kinetics that the cooperativity exists between subunits rather than between binding sites within a subunit (or the Florini and Vestling plots would all proceed through a single point), but precise values for the cooperativity await the completion of equilibrium dialysis studies. The situation is complicated by the fact that both substrate and cofactor seem to take part in the cooperativity. An attempt has been made to fit Hill exponents to the data, but a consistent picture has not emerged, presumably because of this complication.

Table 4 Approximate ratios of K_{AB} to $K_A \times K_B$ for the four enzymes

Enzyme	$K_{ABf}/(K_{\mathrm{EtOH}} \cdot K_{\mathrm{NAD}})$	$K_{ABb}/(K_{\mathrm{Acet}} \cdot K_{\mathrm{NADH}})$
ADH-I	2	1
ADH-II	0.5	1
S-AA-1	1	2
S-AA-5	1	1

It is easy to see how such kinetic alterations would lead to a shift in the reaction towards acetaldehyde, provided that there is a very efficient mechanism for removing acetaldehyde from the cell. The precise magnitude of the shift in the NADH–NAD ratio cannot be determined, since any differences between intracellular compartments are lost in our whole-cell preparations.

Future prospects

These two mutants show specific alterations in function that can be traced to a particular selective pressure. It is likely that the present mutants and others like them can be used to probe the mechanism of cooperative interactions in subunit enzymes. The effect of allyl alcohol selection is to alter the kinetics of one isoenzyme towards an approximation of the kinetics of the other. In neither mutant has any improvement been noted in the velocities of the reactions; a 'better enzyme' has not been produced by these (at the most) two or three mutational steps.

Several questions of evolutionary interest can be approached using this system. Among them are: in how many ways can the molecule be altered to give kinetic changes similar to those seen in these two mutants? What fraction of the molecule has the potential to be involved in this particular adaptive change? Is there a high probability of mutational changes occurring in a particular sequence, or are there many possible sequences? And what is the distribution of amino acid substitutions bringing about these changes? One is not limited here, as is the case with the haemoglobins, to examining a sample of mutations heavily biased towards those that change the charge of the molecule: in this system, it seems highly probable that any alteration in the amino acid composition of the ADH is the result of selection and not of chance.

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letters to nature

Infrared profile of Milky Way at 2.4 μm

THE diffuse component of galactic radiation has been observed in various wavelength ranges, but few observations are yet available in the near infrared range. Since the interstellar extinction is rather weak in this wavelength range, one can obtain an overview of the galactic structure, such as the distributions of infrared sources and of absorbing matter from this region of the spectrum. We have therefore attempted a balloon observation of infrared radiation at the wavelength 2.4 μm with a band width 0.1 μm, avoiding intense OH airglow.

The telescope comprised a silicon of 10 cm (F=0.8) and an array of three PbS detectors attached to the focal plane, each of which had a 3°×3° field of view. The whole optical system was cooled by liquid nitrogen to reduce the background thermal radiation and improve the detector sensitivity. The absolute sensitivity was checked in flight by observing α Her which was simultaneously observed at Agematsu Infrared Observatory of Kyoto University. Relative sensitivity was calibrated in flight by means of calibration light.

The Milky Way was raster scanned in the range $23° \leqslant l \leqslant 75°$ $|b| < 20°$. The isophoto of the infrared surface brightness thus obtained is shown in Fig. 1. The galactic plane was found to be as much as 6° wide (f.w.h.m.) nearly independent of longitude. The surface brightness decreases as longitude increases, as shown in Fig. 2. The flux at the galactic equator is 6×10^{-10} W cm^{-2} μm^{-1} at l=23° and decreases to 8×10^{-11} W cm^{-2} μm^{-1} at l=75°. The longitude dependence of the infrared brightness also shows humps in the tangential directions

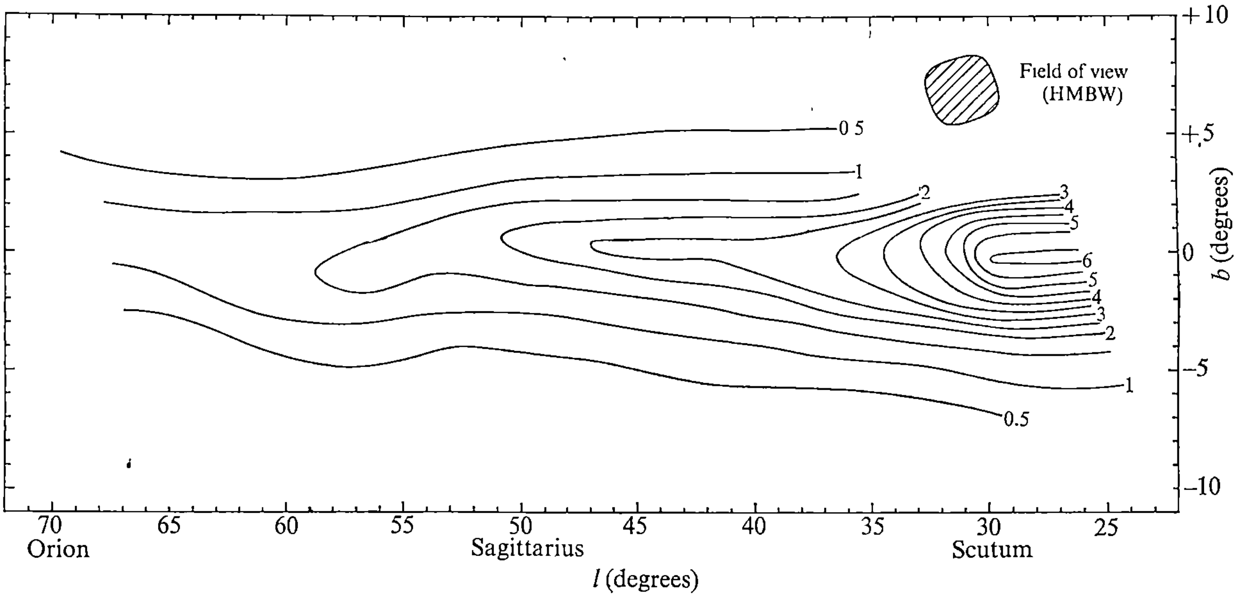
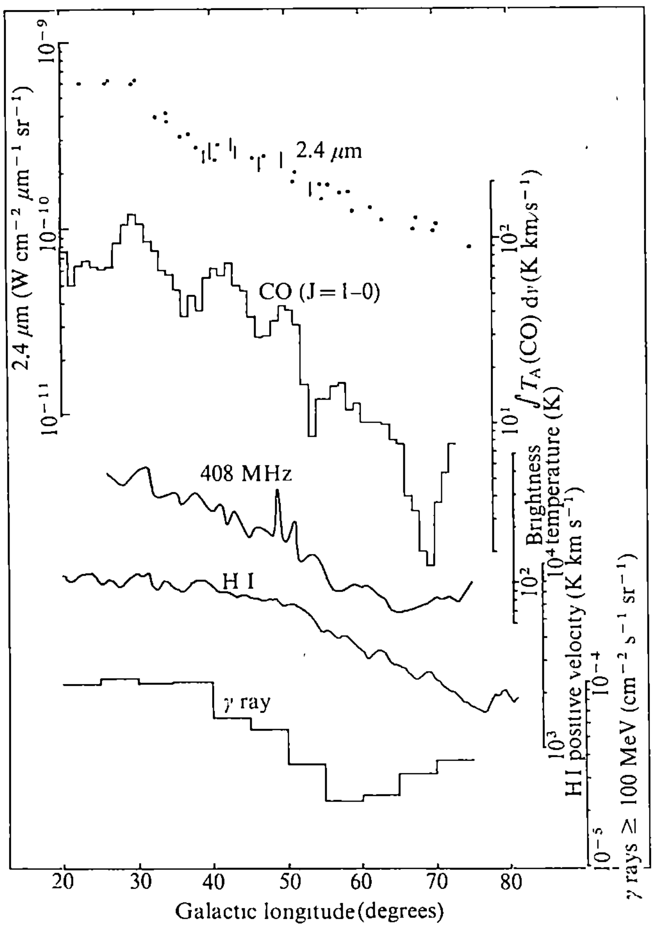


Fig. 1 Isophoto of the 2.4-μm infrared surface brightness The lines are drawn in steps of 0.5×10^{-10} W cm^{-2} sr^{-1} μm^{-1}.

of the spiral arms, $l \simeq 30°$, $50°$. This implies that some fraction of infrared sources are associated with particular objects in the spiral arms.

As a class of such objects, the 2.6-mm line intensity of CO molecules[1] is given in Fig. 2 for comparison. Its longitude distribution shows several peaks at the positions of the infrared humps. Since the CO line intensity represents the surface density of dense molecular clouds, the association of infrared sources with the dense clouds is suggestive. The less steep longitude dependence of the infrared brightness indicates that a considerable fraction of infrared sources is distributed over the Galaxy in a similar manner to normal stars.

Fig. 2 Longitude dependences at the galactic equator of the 2.4-μm intensity (W cm^{-2} sr^{-1} μm^{-1}), the 2.6-mm CO line antenna temperature integrated over velocity (K km s^{-1}), the 408-MHz brightness temperature (K), the 21-cm brightness temperature integrated over positive velocity (K km s^{-1}), and the 100-MeV γ-ray intensity (photons cm^{-2} s^{-1} sr^{-1}).



There may be several other objects which consist of a concentrated component and a diffuse component, the latter showing a distribution similar to general galactic matter. Of those showing longitude dependences similar to the infrared brightness over a considerable longitude range, the intensity of the radio continuum is the most remarkable example. The brightness temperature at 408 MHz (ref. 2) is also shown in Fig. 2. (The brightness temperature at 820 MHz (ref. 3) shows a similar behaviour.) The two spikes seen at ~ $l=50°$ at 408 MHz would have been too sharp to be resolved with our infrared detector. Since the radio continuum is mainly of thermal origin, this correlation indicates an H II region source. In fact, the surface density of H II regions[4] does correlate qualitatively with the infrared brightness.

In contrast to the distribution of H II regions, that of hydrogen atoms shows a much shallower longitude dependence. If, however, only hydrogen atoms with positive velocities are taken into account, the longitude dependence fits exactly with that of the infrared brightness for $l \geq 40°$. The brightness temperature of the 21-cm line integrated over positive velocity[5] is given in Fig. 2. This would suggest that the infrared radiation observed at 2.4 μm is emitted mainly from the region inside the solar circle. A deviation seen for $l < 40°$ may arise partly from the large optical depth (~1 in the low longitude region) so that the column density is not proportional to the brightness temperature.

Finally, comparison is made with the intensity of 100-MeV γ rays[6]. Since the γ rays are produced by collisions of cosmic rays with interstellar matter, their intensity is proportional to the product of densities of cosmic rays and interstellar matter integrated over the line of sight. Thus the distribution is correlated in part with the 21-cm line intensity and also with the density of cosmic ray sources.

It is interesting to see that the longitude dependences observed for the 2.4-μm radiation, the 2.6-mm CO line, H II regions, the 21-cm hydrogen line of positive velocities and the 100-MeV γ rays are, at least in part, correlated with each other. Their interrelationships have been discussed, for example, in connection with the 5-kpc ring, and thus infrared emission provides another means of investigating galactic structure.

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Erosion and the rocks of Venus

THE remarkable photographs of the surface of Venus returned by the Venera 9 and 10 spacecraft have revealed the presence, in two different sites, of a variety of rocklike forms, some angular and some smooth. Press reports[1] express surprise at the absence of very efficient erosional mechanisms. It may be useful to point out instead that it is the presence, not the absence, of erosional mechanisms on Venus which is surprising. The degree of erosion of surface rocks on Venus is determined by an equilibrium between the rate of production and the rate of destruction of surface rocks. The principal causes of erosion of terrestrial rocks—running water, diurnal and seasonal temperature changes, particularly in deserts, and aeolian abrasion—are all absent on Venus. The surface temperature of 750 K is above the critical point temperature of water. Ground based radioastronomical measurements, a comparison of the temperatures measured by Venera spacecraft at a variety of solar zenith angles, together with the high heat capacity of the massive Venus atmosphere, all clearly demonstrate that the diurnal temperature differences are a few degrees K at most[2,3]. The obliquity of the rotation axis of Venus is so small that there are effectively no seasons on the planet. The efficiency of aeolian abrasion depends on the velocity to a power $\geqslant 3$; since both theory and observation show the velocities in the lower atmosphere of Venus to be about an order of magnitude less than at comparable regions in the Earth's atmosphere, it follows that sandblasting on Venus is at most 10^{-3} as efficient as on Earth[4].

The problem is to find a suitable source of erosion of surface rocks—a problem somewhat similar to that raised by the radar discovery of large, presumably impact, basins which, when compared with their lunar, martian and mercurian equivalents, are remarkably shallow. Two mechanisms for the erosion of crater ramparts on the surface of Venus can be suggested[4]; I propose that they may also be important for the erosion of the rocks photographed by Venera 9 and 10. The atmosphere of Venus contains hydrochloric acid at a mixing ratio of $\sim 10^{-6}$, hydrofluoric acid at $\sim 10^{-8}$ and sulphuric acid at larger mixing ratios which, however, are as yet undetermined for the lower atmosphere of the planet[5]. Chemical weathering for lengthy periods by such a mixture of strong acids, even in very dilute concentrations, may be quite adequate to erode angular projections of siliceous rocks.

A second possibility arises from the high surface temperature of Venus. Although these temperatures are not sufficiently high to melt silicates, they are high enough to bring many rather common geochemical materials (for example, NaOH, KOH, HgS and KNO_2) near or to their melting points. If the rocks of Venus are comprised of such materials, even in abundances of a few tenths of a percent, the rheological properties of their low melting point components may, over long periods, be adequate to soften the contours of surface rocks. Deformation of the walls of large basins will occur over long periods, even if the lowest melting point of abundant constituents is considerably above 750 K.

With typical terrestrial values of the subsurface temperature gradient, the high surface temperature of Venus implies that the melting points of silicates should be reached a few tens of kilometres subsurface. The 'granitic' values of the uranium–potassium–thorium radioisotope ratios[6], as determined by Venera 8, suggest that a terrestrial value to the subsurface temperature gradient may be a good first approximation. In this case, access of magmatic material from the interior of Venus to its surface should be considerably easier than on Earth and significant fractions of the surface may be frozen lava fields, having reached thermal equilibrium at the low temperature of 750 K. This may provide both a source of rocks too young to have been eroded and a means of filling large craters and impact basins.

Venera 9 and 10 are the first spacecraft to obtain *in situ* photographs of the surface of another planet and photographs which they have obtained open a new field of very high resolution comparative planetology.

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Airborne ultraviolet studies of Venus

BECAUSE of the high atmospheric extinction in the ultraviolet at ground level, an airborne platform offers distinct advantage for planetary optical measurements in the wavelength region below 3,500 Å. The joint NASA–ESO Space Shuttle simulation programme (ASSESS) provided an opportunity for conducting such measurements aboard NASA's Convair 990 jet aircraft, at an altitude of ~ 40,000 feet. The following summary of these measurements is a supplement to the published report on the ASSESS experiments[1].

The experimental setup for airborne planetary ultraviolet studies consisted of a two-dimensional gyro-stabilised plane mirror (a heliostat) placed near a quartz side window in the aircraft. Objects outside the aircraft could be viewed at elevations between 10° and 40°. A 14″ diameter Cassegrain telescope was fitted with an image position analyser star tracker which fed electrical signals to the movable secondary mirror in the telescope for stabilisation of rapid changes in image position and also to the heliostat to compensate for slow changes that were not compensated for by the gyros of the heliostat. Once an object was acquired, this system automatically tracked it within the elevation and azimuth ranges through which the heliostat could be driven to look at an object through the aircraft window.

Our primary objective was to observe Venus in the ultraviolet region. The telescope image size diameter was ~ 0.5 mm. A quartz lens magnified this to a 5-mm disk in the slit plane of a 1-m Ebert–Fastie spectrophotometer.

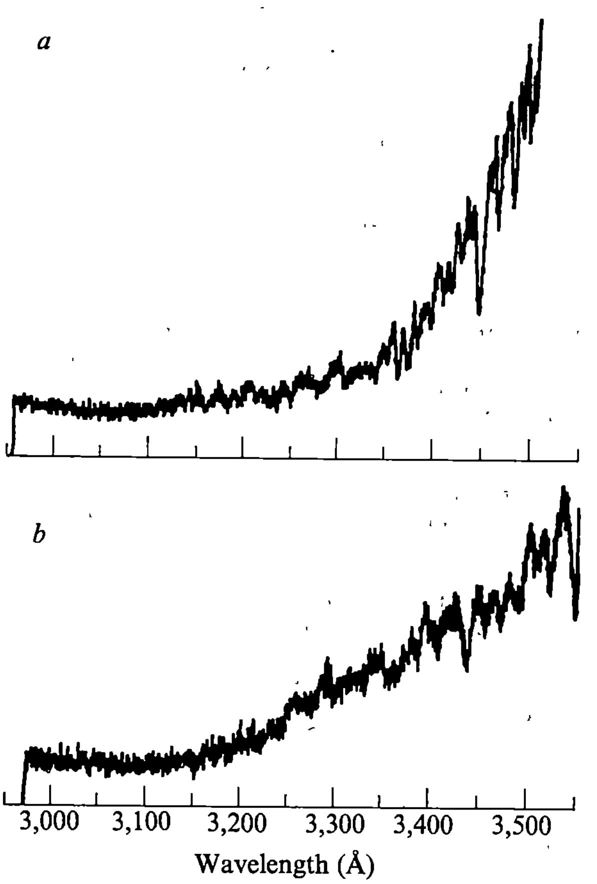

Fig. 1 Solar (*a*) and Venus (*b*) ultraviolet spectra between 2,970 and 3,540 Å at 4 Å resolution.

The primary wavelength region monitored was between 2,950 and 3,350 Å in the third order, with a resolution of ~ 4 Å. A cooled S20 photomultiplier tube fitted with a quartz window, and operating in pulse counting mode, was used as a detector. A mini-computer, a digital multiplexer, a digital tape recorder and an interactive graphic system permitted data organisation for real time analysis and display of the results on a scope and a chart recorder, as well as for magnetic tape recording for subsequent detailed analysis. A D/A converter provided independent records of raw data on both chart and analog magnetic tape recorders. The entire optical and electronic system was adequately automated for single operator control in keeping with the guidelines of the shuttle simulation mission requirements.

Measurements of the solar radiation reflected from Venus were obtained on five flights at elevation angles of 15°–30°. An hour and a half of data were acquired by the experiment operator during the confined portion of the mission and two 0.5-h periods were acquired by the principal investigators later in the programme. Direct solar and lunar spectra were obtained for the same elevation angles and wavelength resolution during two of the flights. Figure 1*a* and *b* illustrates typical solar and Venus spectra. Further analysis requires normalisation of these spectra at one wavelength before obtaining the difference spectrum or ratio spectrum to remove the solar Fraunhofer and terrestrial O_3 absorption features from the Venus spectrum. In a previous set of Venus observations from another expedition using the NASA Convair 990, some absorption features appeared in the wavelength regions of the SO_2 bands. We will analyse our current observations, which are at higher wavelength resolution, to look for conclusive evidence of these bands as well as other features, such as the NH band at 3,357 Å, which might be present.

In addition to these Venus data, we acquired sufficient information to deduce the total O_3 column densities in the Earth's atmosphere. We also monitored the ultraviolet spectra of Jupiter and terrestrial airglow O_2 Herzberg and OH Meinel emissions. In summary, these flights have produced much scientific data which will be helpful to our understanding of the composition of planetary atmospheres. In addition, the evaluation of the simulated shuttle mission should be useful in the planning of scientific missions on board the space lab–shuttle system.

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Glaciations and dense interstellar clouds

McCREA has revived the idea that the Earth's ice ages may have been caused by the interaction of the Sun with dense interstellar clouds[1]. In this theory, originally formulated by Hoyle and Lyttleton[2], the solar luminosity is temporarily increased by the accretion of gas on to the Sun when the Solar System passes through a dense interstellar cloud. The increased insolation is assumed to cause an increase in precipitation and ice accumulation. McCrea suggests that the recurrence and duration of ice epochs can be attributed to the passage of the Solar System through compression lanes in the spiral arms. Relative velocities between the Solar System and the clouds range from 5–20 km s^{-1}, but cloud densities of 10^5–10^7 hydrogen molecules per cm^3 are called for by the model. A study of the character and grain size distribution of texturally mature lunar soils supports this model[3]. Here we examine several consequences of the passage of a dense cloud through the Solar System and find severe problems for this glaciation mechanism.

Since the most recent glaciation ended only $\sim 10^4$ yr ago, we expect to find a dense interstellar cloud at a distance $\sim 1/5$ pc, and in what follows we assume the minimum possible density for the cloud ($\sim 3.3\times10^{-19}$ g cm^{-3}) unless otherwise stated. The duration of the last glaciation was $\gtrsim 7\times10^4$ yr, indicating that the cloud dimensions must be $\gtrsim 1$ pc. Regardless of the relative velocity of the cloud, the solid angle subtended must be nearly 2π steradians, or almost half the sky. Hoyle and Lyttleton acknowledged that their model implied the existence of such a dense nearby cloud, but this could not be ruled out with the observations available at that time (1939).

It is now straightforward to eliminate the possibility of the cloud being atomic or ionised hydrogen. The optical depth in the 21-cm line of atomic hydrogen is $[n/(4\times10^{18}\,T_s)]\Delta X$, where ΔX is the cloud thickness, n is the number density of hydrogen atoms, and T_s is the spin temperature. For a reasonable value of the spin temperature of 100 K, we obtain $\tau_{\mathrm{HI}}\gtrsim 750$. If the cloud is completely ionised, then the optical depth from free–free transitions is

$$9.8\times10^{-21}\,\frac{n^2\Delta X}{\nu^2 T_e^{3/2}}\ln\left[49.5\times10^{-3}\,\frac{T_e^{3/2}}{\nu}\right]$$

where ν is the frequency in GHz, and T_e is the electron temperature[4]. For $T_e\sim10^4$ K and $\nu=1$ GHz, we have $\tau_{\mathrm{HII}}\gtrsim 2{,}000$. Clearly, no significant fraction of the cloud mass can be in the form of atomic or ionised hydrogen.

The usual possibility referred to[1,2] is that the cloud consists

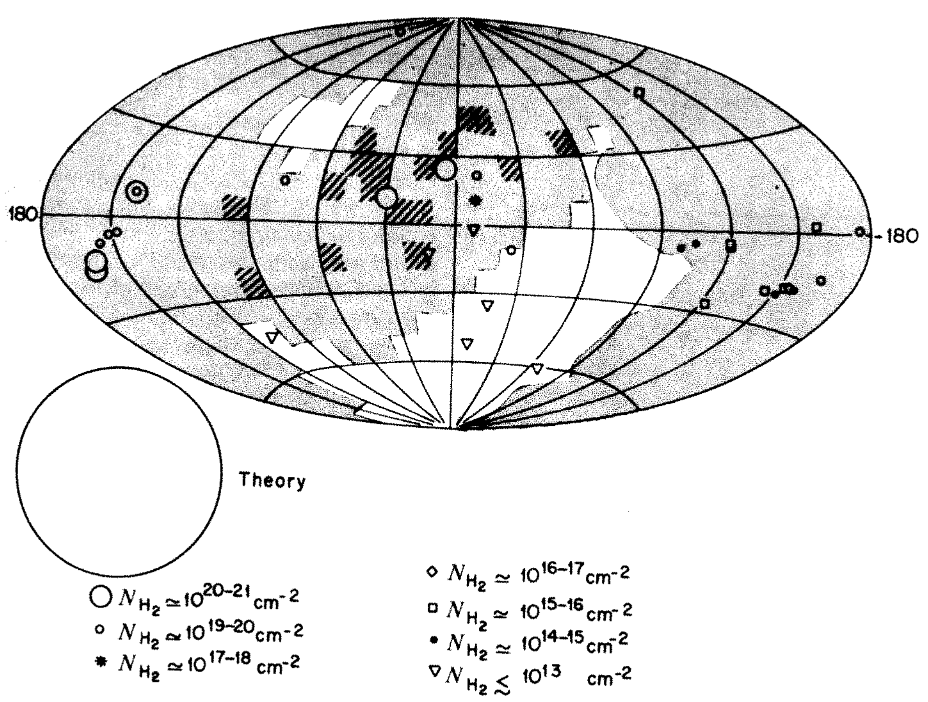
180
-180
Theory
○ $N_{H_2} \simeq 10^{20-21}$ cm^{-2}
∘ $N_{H_2} \simeq 10^{19-20}$ cm^{-2}
✱ $N_{H_2} \simeq 10^{17-18}$ cm^{-2}
◇ $N_{H_2} \simeq 10^{16-17}$ cm^{-2}
□ $N_{H_2} \simeq 10^{15-16}$ cm^{-2}
• $N_{H_2} \simeq 10^{14-15}$ cm^{-2}
▽ $N_{H_2} \lesssim 10^{13}$ cm^{-2}

Fig. 1 Equal area projection in galactic coordinates of collected observations of the soft X-ray background, and column densities of H_2 obtained from stellar ultraviolet absorption spectra. The soft X-ray background has been observed in shaded regions, with upper limits obtained in hatched regions. X-ray observations are not available in the clear areas. Symbols show column densities of H_2, and for those cases in which $N_{H_2} > 10^{19}$ cm^{-2}, the areas of the circles are proportional to the column densities. The column density predicted by the model for approximately half of the sky is illustrated by the large circle.

of molecular hydrogen. Indeed, an H_2 cloud of such density would not require a significant proportion of dust for shielding since it would primarily shield itself against ambient ultraviolet radiation[5]. In addition, the postulated cloud is completely optically thick to X rays with energy $\lesssim 3$ keV (ref. 6). In Fig. 1 we have combined published observations at 0.28 keV of the soft X-ray background[7-9]. At this energy the optical depth of the cloud is $\gtrsim 1{,}300$. Superimposed on these observations we depict column densities of interstellar H_2, derived from satellite observations of H_2 absorption lines in the ultraviolet spectra of selected stars[10,11]. The largest column density reported from the ultraviolet measurements is $N_{H_2} \simeq 4 \times 10^{20}$ cm^{-2}, whereas the model calls for $N_{H_2} \geq 2 \times 10^{22}$ cm^{-2}, with $N_{H_2} \simeq 4 \times 10^{23}$ cm^{-2} being a more typical value. From the lack of extensive absorption of the soft X-ray background, and the lack of sufficient H_2 column densities, we can rule out the existence of such a dense molecular cloud of large angular size.

The observational constraint that the cloud be transparent to visible light implies that for a cloud of solid particles the mean free path for mutual collisions is comparable with, or greater than, the cloud dimensions. Such a cloud cannot be appreciably accreted on to the Sun since mutual collisions are required to destroy the systematic velocity of the cloud material as it sweeps behind the Sun. Thus, the proposed cloud could not consist of dust.

The transparency to visible light also implies that, for micron-sized dust grains, the dust–gas mass ratio $< 10^{-4}$. This is at least 2 orders of magnitude lower than the usual value.

We now consider the dissipation of the orbits of small particles in the Solar System, because of the relative motion between the particles and the cloud. Neglecting the initial velocity of the cloud relative to the Solar System, this force will significantly disrupt the orbits on a timescale:

$$t \simeq l(\rho/\rho_c)(a/GM_\odot)^{1/2}$$

where l is the radius of a particle, ρ is the mass density of a particle, ρ_c is the cloud density, and a is the orbital radius For the zodiacal dust cloud we take $a \simeq 1$ AU, and $l \simeq 1$ µm, and obtain $t \simeq 4$ yr—even centimetre-sized particles would have been removed from the Solar System during the last glaciation. At present, much weaker dissipation mechanisms are operative, and the replenishment of the zodiacal dust cloud requires $\gtrsim 10^5$ yr (ref. 8) as against the $\sim 10^4$ yr since the last glaciation.

The possibility of replenishment by the dense cloud itself cannot be dismissed. Any such consideration must, however, account for the formation of the planar distribution of the zodiacal dust since the last ice age and the remarkable coincidence of the plane with the ecliptic.

We also find that this same dissipative force can remove particles of ~ 1 cm from Saturn's rings during the $\sim 7 \times 10^4$-yr glaciation period. Pollack *et al.*[9] interpret existing observations of the rings to indicate a mean particle radius of ~ 1 cm. They hypothesise that small particles are continually created through meteoroid impacts. Particles considerably smaller than 1 cm are quickly removed by the Poynting–Robertson drag.

The McCrea model links purportedly periodic glacial phenomena with regular astronomical occurrences. The constraints discussed present grave difficulties in explaining at least the last glaciation in this way. These constraints, as pointed out by McCrea, are of interest since the Solar System must occasionally pass through interstellar clouds.

Another possibility is that this mechanism acts merely as a rapid trigger for a longer lived glacial episode. This tends to complicate the conjecture regarding the climatic phenomenon somewhat. The observational data constrain the angular size of the cloud $\lesssim 30°$, indicating that the ice must persist for $\gtrsim 5$ times the trigger duration. In this case, the optical depth of the cloud, though still severe, is reduced by a factor of five. X-ray observations in those directions not yet examined will probably restrict the possibilities even further. Although the smaller cloud would wipe out the zodiacal dust in ~ 20 yr, the zodiacal dust would have had a longer time for replenishment. The 6×10^4 yr since its passage is a sizeable fraction of the replenishment time.

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Proterozoic oceanic crust at Bou Azzer

THE precise nature of the last great Precambrian orogeny (~600 Myr BP) is the subject of controversy, and views diverge as to whether it was a collision orogeny[1–3] or whether *in situ* deformation of an intracratonic orogenic belt occurred[4–6]. Geological evidence is given here, indicating the former presence of an oceanic domain along the northern margin of the West African Craton.

The Precambrian basement of the Anti-Atlas comprises two major structural units: the northern border of the West African Craton to the south-west, and a portion of the Pan-African Orogenic Belt to the north-east (Fig. 1). The linear Bou Azzer Complex[7], composed of ultrabasic to acid, plutonic and volcanic rocks in a greenschist metamorphic environment, is located along the edge of the craton in the central part of the Anti-Atlas. Younger than the craton, which stabilised around 1,800 Myr BP, it was affected by Pan-African metamorphism (~ 640 Myr BP).

A vertical section, 4–5 km thick, the Bou Azzer Complex comprises, in ascending order: upper mantle peridotites (harzburgites, dunite with some diallagites), ultrabasic cumulates (dunite–werhlite–clinopyroxenolite sequences), basic cumulates (layered gabbros), large stocks of quartz diorite, submarine lavas (diabases and spilites often displaying pillow structures) overlain by spilites, keratophyres, quartz keratophyres, and tuffs accompanied by greywackes, siltstones, limestones and jaspilites (Fig. 2). All the ultrabasic rocks have been serpentinised, in several stages; so the contact between residual mantle peridotites and overlying utrabasic cumulates is not clearly defined. Ultrabasic and basic cumulates are the result of evolution in a magmatic chamber under a roof of tholeiitic basic lavas, in a shallow and hydrated environment undergoing distension, as shown in the layered gabbros by presence of magmatic breccias, diabases, and keratophyre sills in the lower part, which fed dyke swarms at a higher level. At the top of the layered gabbros, beneath the tholeiitic lavas, intrusion breccias comprising angular fragments of diabase enclosed in leucogabbro occur. Most of the quartz–diorite stocks, displaying flaser structures, are located between the gabbros and the overlying basic lavas. Late quartz diorites have been intruded syntectonically into keratophyres, with resulting contact metamorphism.

The Bou Azzer Complex is a typical ophiolite[8] assemblage displaying the same rocks facies, the same vertical sequence, and the same thickness as those of the classical Palaeozoic (Ballantrae, Newfoundland) and Mesozoic (Oman, Troodos, Vourinos) suites. In accordance with current concepts of ophiolite as oceanic lithosphere[9–11], the Bou Azzer Complex is here interpreted as a sheet of allocthonous, Proterozoic oceanic lithosphere.

During the major phase of folding of the Pan-African Orogeny, which produced recumbent isoclinal folds towards the WSW and greenschist metamorphism, two palaeogeographical domains collided. The oceanic crust (ophiolite) was thrust, locally in an inverted position, over the northern border of the West African Craton, itself characterised by epicontinental limestones, quartzites and basic lavas with alkaline trend.

Also in the Pan-African Belt, 2,000 km from the Anti-Atlas, on the eastern edge of the West African Craton, in the Adrar of Iforas (Mali), disturbed ophiolite (upper mantle metaperidotites, gabbros, basic lavas, keratophyres, tuffs, jaspilites) are obducted into an epicontinental sedimentary unit.

The discovery along the margins of the West African Craton of typical ophiolites, interpreted as vestiges of Middle or Upper Proterozoic spreading oceanic crust, shows that a model based on plate tectonics, not necessarily of

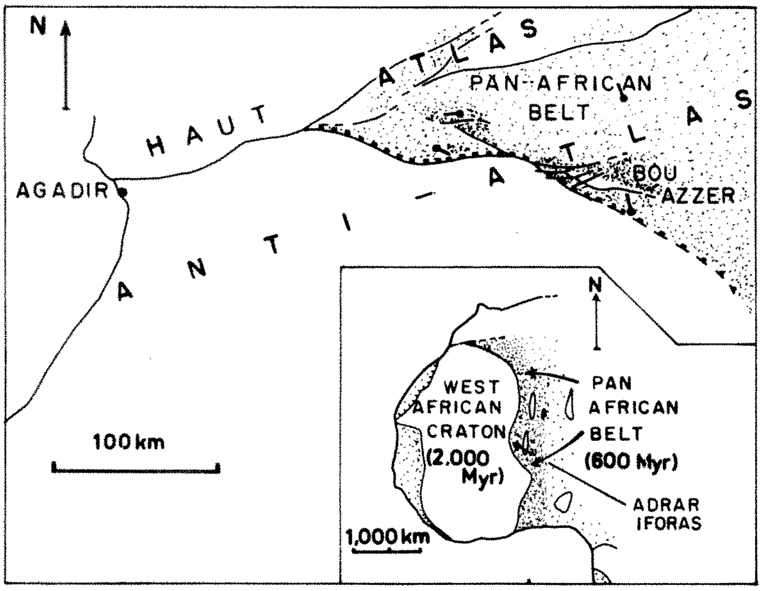

Fig. 1 West African Craton and Pan-African Belt, north-western Africa. Broken line, northern edge of West African Craton.

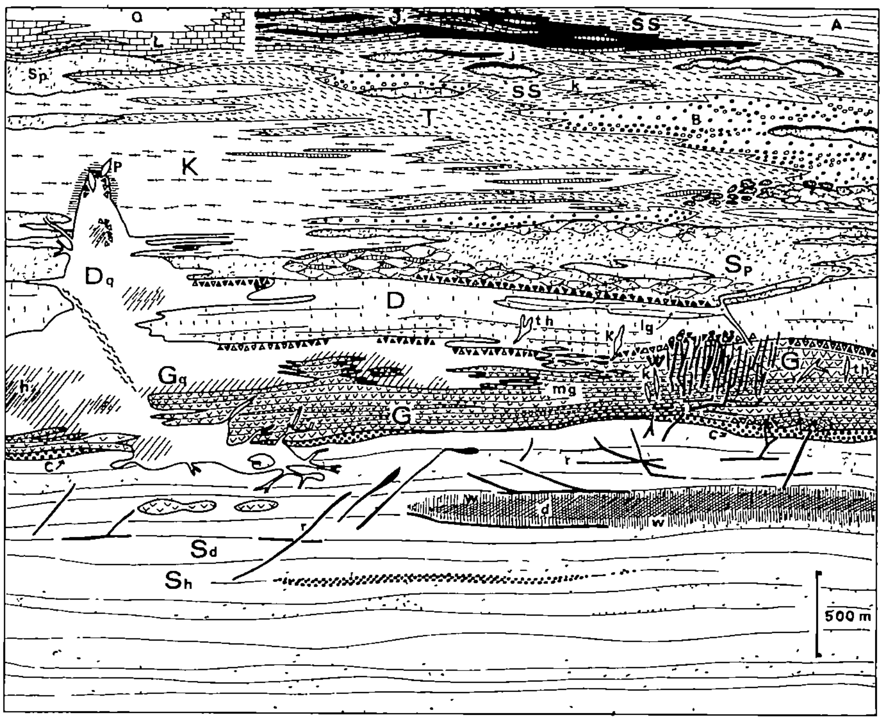

Fig. 2 Schematic section of the Bou Azzer ophiolitic complex. S_h, S_d, Serpentinised harzburgites and dunites with (d, w) level of diallagite and wehrlite and (r) dykes of rodingitised gabbros; C, ultrabasic cumulates; G, layered gabbros; W, swarms of basic dykes; th, dykes of trondhjemite; mg, microgabbros; d, diabases; D_q, quartz diorites; G_q, quartz gabbros; h, hornblendites; p, intrusive apex, Sp, spilites; K, keratophyres; T, tuffs; j, jaspers; B, greywackes; SS, siltstones; L, limestones, J, jaspilites; Q, sandstones; A, arkoses.

large scale, can be applied to the Pan-African Orogenic Belt.

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Preparation and properties of rubber dislocations

The purpose of this note is to describe the construction and properties of a Volterra[1] edge dislocation made from two sheets of smooth rubber. A simple theoretical analysis illustrates the key features of the model and illuminates its interesting and unexpected behaviour.

A smooth transparent rubber sheet was manufactured by cross linking ethylene–propylene copolymer against a glass plate in a heated press[2,3]. After cutting the rubber into strips and removing it from the glass, two smooth pieces were brought into contact to form a laminate containing a single reversible adhesive interface (Fig. 1*A*). The Volterra dislocation of Burgers vector *b* was then formed by peeling one end of the laminate, displacing it rigidly, and re-adhering at the interface (Fig. 1*B*)[4]. Clearly, dislocations can exist in an essentially amorphous material[5,6].

Viewed in the geometry of Fig. 1*B* the vital features of the dislocation are obvious! The dislocation is composed of two, opposite facing cracks, each capable of moving reversibly along a special weak plane in the body. These cracks differ from conventional cracks in that they can heal, where the surfaces come close together, to produce an interface comparable in strength with that before fracture. Other close similarities between cracks and dislocations have been noted[7,8].

I now consider the condition for the existence of the dislocation. The dislocation persists in spite of the attractive surface forces continually striving to close the cracks and collapse the structure. These attractions are counteracted by elastic forces in the bent rubber. In equilibrium, the dislocation reaches a length *z* which can be calculated using a simple energy argument.

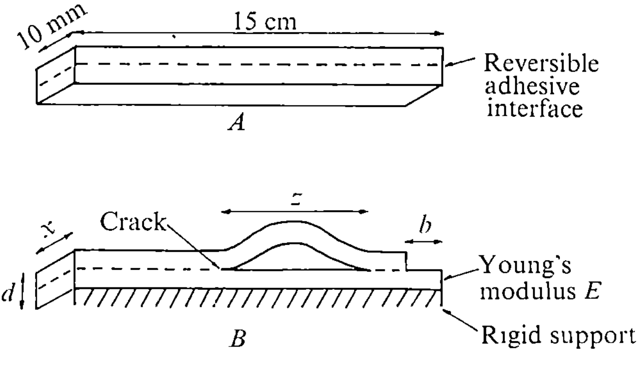
The free energy of the rubber surfaces is zRx where R, the interfacial energy, is the work needed to separate unit area of the interface. The elastic energy in the bent strip, treated as a strut collapsing at constant force[9], is approximately $\pi^2Exd^3b/3z^2$ using the nomenclature of Fig. 1. Therefore, by energy conservation, neglecting crack tip strains which are assumed to remain constant

$$\frac{d}{dz}\left(zRx + \frac{\pi^2Exd^3b}{3z^2}\right) = 0 \qquad (1)$$

$$z = \left(\frac{2\pi^2Ed^3b}{3R}\right)^{1/3} \qquad (2)$$

This equation, giving the size of the hole in a Volterra dislocation, bears comparison with that due to Frank[10] who suggested that such holes should be small. Peierls[11] also suggested that

Fig. 1 *a*, Rubber laminate with a single reversible adhesive interface; *b*, Volterra edge dislocation in the rubber laminate.



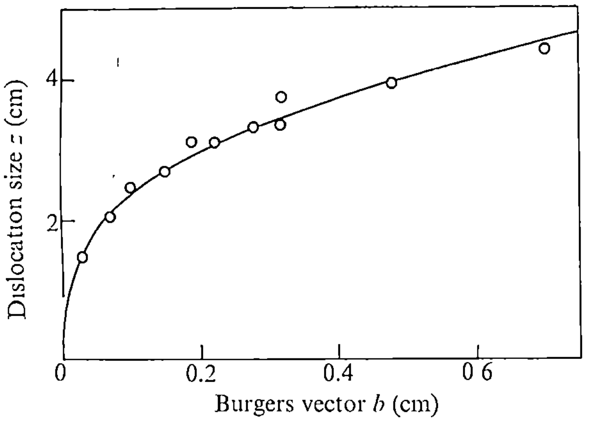


Fig. 2 The size of a rubber dislocation.——, Equation (2) with $R = 0.57\ \mathrm{J\,m^{-2}}$.

dislocations should be small using the enigmatic argument, "... we were not actually justified in our fundamental assumptions, which took the extension of the dislocation to be large. But the dislocation cannot, in any case, be much larger than we have found, since the equations ought to be correct if it were."

The dislocations of my experiments had big holes, as shown by the results of Fig. 2. To obtain these results, a dislocation of desired Burgers vector was partially closed by applying pressure at the cracks. These cracks opened up spontaneously on releasing the pressure however, and the dimension z was measured after 10 min. Equation (2) describes the results reasonably well if the interface energy R is taken to be 0.57 $\mathrm{J\,m^{-2}}$, about that measured in peeling tests at similar crack speeds.

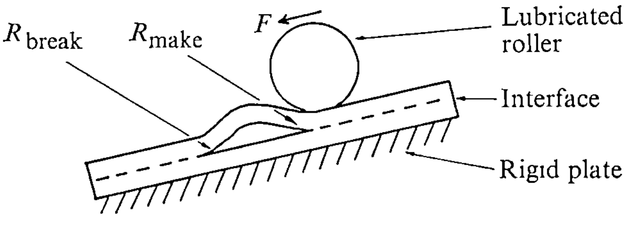
The Volterra dislocation could also be moved along the interface using a talc-lubricated roller (Fig. 3). The crack at the front of the dislocation then opened continuously and that at the rear closed. A propagation criterion was derived by considering the work done by the applied force F against the surface forces at the crack tips. If the energy expended in cracking is R_{break} per unit area, and the energy gained in reforming the interface is R_{make}, then

$$F = x(R_{\mathrm{break}} - R_{\mathrm{make}}) \qquad (3)$$

This equation, supported by experiment, shows that resistance to dislocation motion arises by interfacial hysteresis[12], akin to contact angle hysteresis, where energy is dissipated in breaking and remaking an interfacial bond. A similar equation describes the rolling friction of a cylinder on a smooth surface[13,14].

A stimulating phenomenon observed in these experiments was dislocation disappearance. The dislocation became shorter as it travelled, finally disappearing altogether. This was seen to be a result of the shedding of strain energy. Rubber deposited behind the moving dislocation was not unstrained, but was under slight compression. Thus the moving dislocation left some of its strain energy behind, gradually disappearing in the process. Another way of making the dislocation disappear was to deform the laminate so as to bring the sheets of rubber together across the dislocation. Stretching or bending the laminate did this; the dislocation healed completely and was reluctant to reappear.

Fig. 3 Dislocation movement



Equations (2) and (3) gave a rather oversimplified view of the behaviour of the dislocation. The surface energy of the rubber was not a constant but varied over several orders of magnitude with crack speed (both making and breaking)[12], with temperature, and with time of interfacial contact. Equilibrium could not, in fact, be achieved because of adhesive hysteresis[13].

Such non-equilibrium effects have not been taken into account in previous dislocation theories, yet they produce some amusing demonstrations. For example, the dislocation travelled at constant speed under steady load but increased in speed and became shorter as the driving force was raised; the dislocation moved faster as the temperature was increased; a second dislocation moved faster than the first; two similar dislocations repelled each other and resisted merging. If sufficient force was applied however, the dislocations joined to form a large dislocation.

These observations may be of value in explaining the deformation of materials, both crystalline and amorphous. In addition, some curious properties of composites[15] and frictional contacts[16–18] might be clarified.

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Creeping-film phenomenon of potassium chloride solution

A PECULIAR creeping film that is similar to the superfilm of liquid helium II, but which occurs at room temperatures and has a much lower transfer rate, takes place in saturated potassium chloride aqueous solution. In an electrolytic experiment using saturated KCl solution as the salt bridge, we noticed that without any disturbance the solution could not be kept in the beaker all the time. A thin surface film creeps up the beaker wall very slowly, about several centimetres a day, flows out over the brim and then down the outside surface of the beaker. Creeping can also be observed up the surface of the electrode and the salt bridge tube (Fig.. 1). On surfaces over which the film has crept, potassium chloride crystals form as the water in which KCl is dissolved evaporates. Within a few days the solution in the beaker drains out completely and the entire surface of the beaker becomes covered with a thin layer of potassium chloride crystals, about 10^{-2} cm thick.

Another anomaly is the leakage of the same liquid solution from a supposedly 'seamless' glass tube connector used for the connection of the salt bridge. The solution flows out through the 'seamless slit', and then spreads all over the outside surface (Fig. 2). The creeping film then evaporates, and crystals form. The creep rate is lower than the previous case. The phenomenon occurs when the connector is

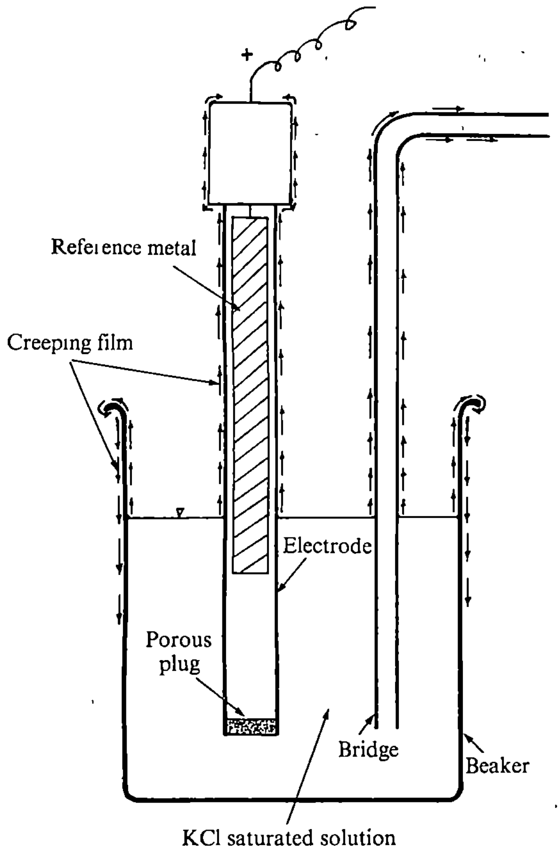
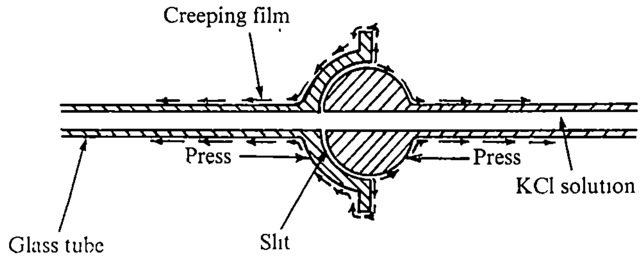


Fig. 1 Surface creep in a set-up including an electrode and a salt bridge.

pressed with a clamp so tightly that water or air, even at very high pressures, cannot pass through.

An experiment was carried out to investigate whether this creeping thin film resulted from the electrical effect of the electrode. Experiments similar to the surface film experiment for liquid helium II were carried out. Instead of the electrode and the salt bridge, two small glass tubes, 1.8 cm in diameter and 11 cm long, were filled with 4 cm and 7 cm, respectively, of saturated KCl aqueous solution and immersed in two 400-cm^3 beakers containing 300 cm^3 of same KCl solution. The glass tubes were immersed in such a way as to provide a difference in level of 3 cm between the liquid in the tube and that in the beaker. To investigate the possible effect of the difference in level, experiment A was performed with the liquid in the tube higher than that in the beaker (Fig. 3). In experiment B the level was lower in the tube. The experiments were carried out with the surface as clean as possible so as to avoid contamination. No observable disturbance was allowed during the experiments. Flow of the creeping film along the surface of the beaker and along the inner and outer surfaces of the tube was observed, with a transfer rate of several centimetres a day.

Fig. 2 Flow through a 'seamless slit' connector.



The film thickness was estimated to be 10^{-2} cm from observation of crystal thickness.

The creeping rate of the liquid inside the tube in experiment A was, however, higher than that observed in experiment B. About two weeks later, the flow spread all over the inner and outer surfaces of the beaker, and started to extend across the bench until the solution had completely drained out. The solution also ascended the tube surface and crept along the supporting string. It is interesting that dried crystals accumulated at the entrance to the tube.

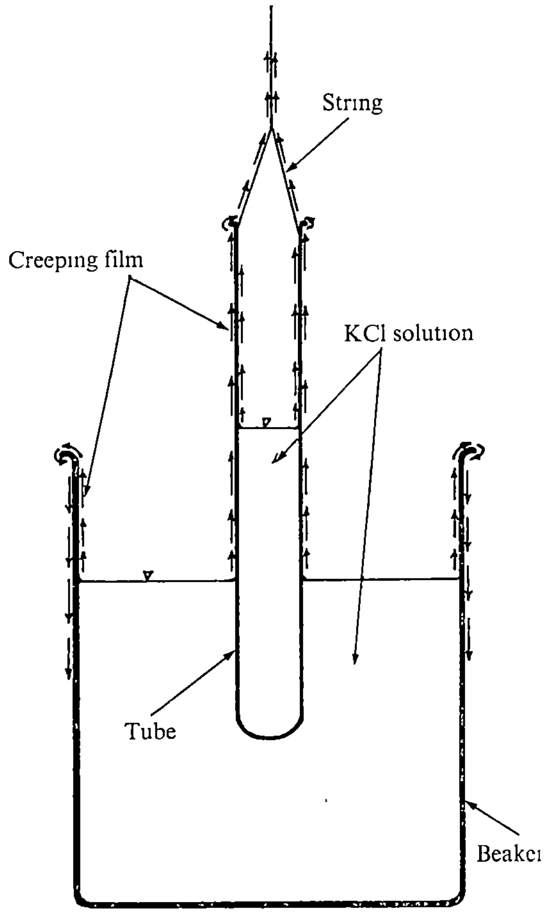


Fig. 3 Experimental set-up without electrode.

After all of the solution in the beaker had drained out, we observed that the heavily accumulated crystal formed a helical shape on the outer wall of the tube and the inner wall of the beaker.

The temperature was maintained at about 22 °C, though slight fluctuations were inevitable. Humidity was not controlled during the experiments. It seems that humidity as well as surface conditions were responsible for the creeping rate of the film. We repeated the experiments using a 'wet' surface, and the transfer rate increased by a factor of about three. But we could not reproduce the experiment from run to run even by using very clean surfaces. It seems that the creep is unlikely to occur if the solution is not saturated (this can happen if the temperature rises so that the solution becomes unsaturated).

This surface film phenomenon cannot be explained in terms of the evaporation of the solution from the bulk liquid level with subsequent condensation on to the surface, because the film creeps over the brim of the beaker. The experiments showed that the phenomenon is not attributable to any electrical effect although that may play a rôle if an electrode is present. No satisfactory interpretations based on present knowledge of either surface chemistry or transport theory can be given. The superfilm phenomenon of liquid helium II seems similar to the phenomenon described here; however, potassium chloride solution has entirely different physical and chemical properties from helium II.

We attribute this phenomenon to the interactions of the ions with the surface of the beaker or tube and the surrounding gaseous molecules; the major driving force is not clear. The leakage through a tightened glass connector is also inexplicable.

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Radiometric age of early *Hipparion* fauna in North-west Africa

A TUFF for which a $^{40}Ar/^{39}Ar$ date of 12.18±1.03 Myr has been obtained is located in the lower level of the Bou Hanifia continental formation (= Oued el Hammam), several metres below the famous *Hipparion primigenium* locality[1–3]. The tuff forms a continuous layer in the lower part of the continental formation and can be observed on either side of the Oued el Hammam Dam. The *Hipparion* fossiliferous horizon (Bou Hanifia I) lies in a second deposit of greatly weathered tuffs and has yielded a few micromammal remains. About 15 m above Bou Hanifia I, a second micromammal locality has been discovered (Bou Hanifia II)[4].

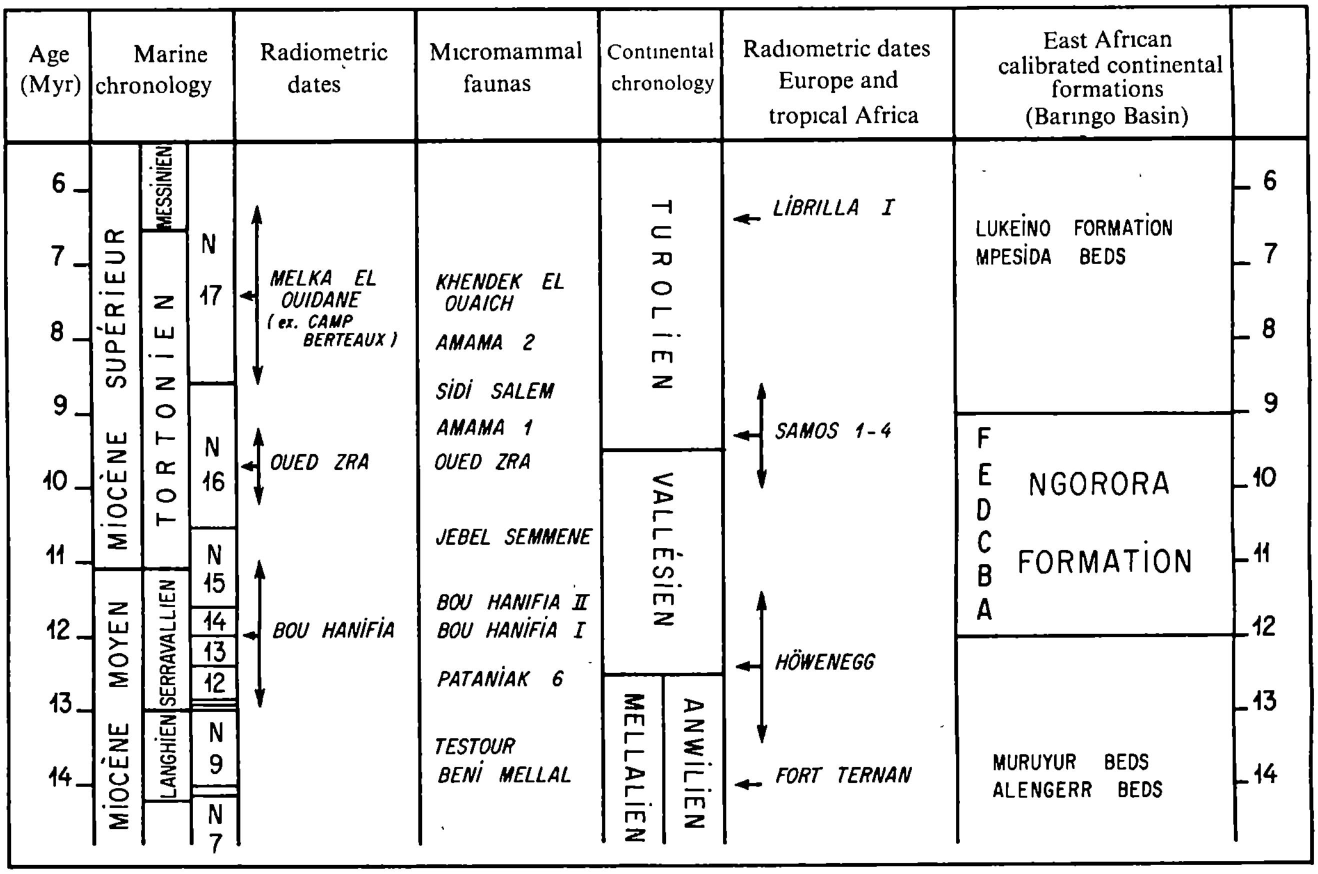
The faunal lists are as shown in Table 1.

Table 1 Faunal lists for Bou Hanifia I and II

Bou Hanifia I	Bou Hanifia II
Hipparion primigenium (Von Meyer) *Dicerorhinus primaevus* Aramb. *Orycteropus mauritanicus* Aramb. *Palaeotragus germaini* Aramb. *Samotherium* sp. *Damalavus boroccoi* Aramb. *Gazella praegaudryi* Aramb. *Percrocuta algeriensis* (Aramb.) *Propotamochoerus devauxi* Aramb. Proboscid. indet. (*Deinotherium*?) *Progonomys cathalai* Schaub *Myocricetodon* sp. *Testudo*? *Struthio* sp.	*Zramys* cf. *dubius* Jaeger *Progonomys cathalai* Schaub

The Bou Hanifia Formation, about 400 m thick, lies unconformably on a lower Miocene (Cartennian) formation[1,2], and is overlain by a marine formation assigned to the N17 Zone of Blow[5]. The transition from continental to marine horizons can be observed 6 km to the south of the dam along the N7 road from Sfisef to Ain Fekan, beyond the 'three rivers' bridge. A third micromammal deposit (Sidi Salem) has been discovered there directly below some intermediate brackish deposits with *Potamides* and oysters[4]. Its fauna closely resembles that of the Oued Zra which is 9.7 Myr old, but it contains more highly evolved species. The Sidi Salem deposit is more recent than 9.7 Myr, and is most probably between 9 and 8 Myr old. Thus, the Bou Hanifia Formation covers a time span of around 12–9.7 Myr BP.

Table 2 Relationships of Middle and Upper Miocene micromammal faunas from northern Africa to marine chronology, radiometric dates and continental chronology



Age (Myr)	Marine chronology			Radiometric dates	Micromammal faunas	Continental chronology		Radiometric dates Europe and tropical Africa	East African calibrated continental formations (Baringo Basin)	
6	MIOCÈNE SUPÉRIEUR	MESSINIEN	N 17			TUROLIEN		LIBRILLA I		6
7		TORTONIEN		MELKA EL OUIDANE (ex. CAMP BERTEAUX)	KHENDEK EL OUAICH				LUKEINO FORMATION MPESIDA BEDS	7
8					AMAMA 2					8
9			N 16		SIDI SALEM AMAMA 1			SAMOS 1-4	F E D C B A NGORORA FORMATION	9
10				OUED ZRA	OUED ZRA	VALLÉSIEN				10
11	MIOCÈNE MOYEN	SERRAVALLIEN	N 15		JEBEL SEMMENE					11
12			14 13	BOU HANIFIA	BOU HANIFIA II BOU HANIFIA I					12
13			12		PATANIAK 6	MELLALIEN	ANWILIEN	HÖWENEGG	MURUYUR BEDS ALENGERR BEDS	13
14		LANGHIEN	N 9		TESTOUR BENI MELLAL			FORT TERNAN		14
			N 7							

The period covered by the formation, its relationships with the marine formation, its mammal fauna and the radiometric dates obtained, enhance its importance in the study of the Middle and Upper Miocene of Africa.

Several conclusions can be drawn from the date obtained for the Bou Hanifia tuff.

- The immigration of *Hipparion primigenium* is contemporary in northern Africa and western Europe[6]. The oldest *Hipparion* (*H. primigenium*) known in tropical Africa[7] is slightly more recent than those of northern Africa and Europe. (The meaning of this heterochronism is not yet understood.)
- The immigration of the primitive Murid, *Progonomys cathalai* Schaub, is synchronous with that of *H. primigenium* in the Mediterranean Basin; this has been postulated already[8], but no radiometric date has been obtained before for a fauna including an association of the two species.
- The pre-Vallesian age of the Beni-Mellal fauna is confirmed[4] establishing that it is more or less contemporaneous with that of Fort Ternan in Kenya[9]. On that basis the association observed in Tunisia of Upper Langhian foraminifers[10] with micromammals similar to those of Beni-Mellal[11] seems explicable[10].
- The dates complete the biochronological sequence calibrated with rodents of the micromammal localities of the Middle and Upper Maghrebian Miocene (ref. 4, and Table 2).

Table 2 requires further comment:

- *Hipparion primigenium* persists in northern Africa through the Turolian (Melg el Ouidane Deposit). The species is known from around 4 Myr BP in eastern Africa[12].
- *Hipparion sitifense* Pomel from the Sétif[13] and Mascara[14] lacustrine limestones has been found from the Upper Miocene of Algeria (Amama 2 deposit) and Kenya[16]. So an Upper Pliocene age for the Mascara and Sétif limestones cannot be considered as definitively established.
- An initial conventional K/Ar date of 7.84 Myr was first obtained for the Bou Hanifia tuff. This date contradicted the age indicated by the presence of the Bou Hanifia rodents which are less evolved than those of the Oued Zra deposit (9.7 ± 0.5 Myr) and did not tie in with the more primitive character of the Bou Hanifia large mammals comparable to those of the oldest Samos localities (Samos 1–4), for which a date of 9.3 Myr years has been obtained[17]. Here again, the evolution of rodents provides a particularly dependable tool for the elaboration of continental biostratigraphic scales. The interpretation of the full ^{40}Ar/^{39}Ar age spectrum analysis of biotite from the Bou Hanifia tuff is that it was altered during early cementation/diagenesis around 12.18 ± 1.03 Myr BP; the age of sedimentation must have been a little earlier.

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Orientation specific colour adaptation at a binocular site

MCCOLLOUGH[1] found that after alternate viewing of vertically oriented red gratings and horizontally oriented blue gratings, achromatic gratings appear tinted blue when oriented vertically and pink when oriented horizontally. This indicates the existence of colour specific edge detectors in the visual system. Further, since interocular transfer of the effect was not possible, McCollough concluded that no binocular cells are involved in this type of adaptation. This view has been supported by many investigators[2–5] who used different paradigms. But their failure to illustrate orientation-contingent colour adaptation at a binocular locus may be attributable to the fact that most of their experimental situations, like McCollough's, did not provide the ideal stimulus for binocular neurones, which respond best, showing "binocular synergy", only when the stimulus is simultaneously presented to both eyes[6–9]. I have attempted optimal excitation of the binocular cells by alternately presenting red vertical and blue horizontal gratings to both eyes and isolating adaptation of binocular neurones by cancelling monocular adaptation with presentation of a pair of complementary gratings (blue vertical and red horizontal) to each eye separately for an equal length of time. The results of this procedure show that adaptation does involve binocular neurones.

If only the monocular channels are colour coded, no aftereffect should be obtainable on viewing an achromatic test pattern, since the group of monocular neurones specific to any of the two orientations have been presented with both the complementary colours for equal periods of time. On the other hand, if any of the binocular orientation detectors are colour contingent, an aftereffect complementary to the binocular presentation, namely, an achromatic test grating appearing bluish when vertical and pinkish when horizontal, should be seen on viewing the test stimulus with both eyes. I am aware of MacKay and MacKay's argument[10] against selective adaptation of single units sensitive to colour and orientation, but since it is not contradictory to my central theme I shall talk of "selective adaptation of cells", and leave discussion of their hypothesis for the final interpretation.

Coloured rectangular gratings (dark : light = 1 : 2) of 2.5 cycles per degree, subtending a visual angle of 15° at a distance of 8 feet were presented to five naive observers with normal form and colour vision in the following sequence (colour filters used were Wratten Nos 25 and 44): red vertical, binocular; blue vertical, right eye; red horizontal, right eye; blue horizontal, binocular; red horizontal, left eye; blue vertical, left eye. Each of the above presentations was for 8.5 s with a dark interval of 1.5 s between successive presentations, and the whole cycle was repeated for a total duration of 30 min. When these observers were later shown a test pattern containing achromatic gratings of both vertical and horizontal orientations, all of them reported seeing bluish vertical and pinkish horizontal gratings when both the eyes were open; with either eye occluded they all reported seeing pinkish vertical and bluish horizontal gratings. No leading questions were put to them and their choice of colour tints on viewing the test stimulus was always spontaneous. The aftereffects could be easily obtained up to 24 h after the adaptation, comparable to the time-course of the classical McCollough phenomenon.

These observations point to the colour specificity of two types of binocular cells: (1) cells which respond much more strongly for a binocular stimulus than for a monocular one. Electrophysiologically, most binocular units have been shown to be of this type[6-9], though their colour specificity has not been clearly demonstrated. (2) Neurones which receive a facilitatory input from one eye and an inhibitory signal from the other. The simple cells in area 17 reported by Hubel and Wiesel to be monocularly driven[6,11] have been shown[12] to receive a binocular input in the cat. Though these cells are excited only through the "dominant" eye, an "incorrectly" placed stimulus in the non-dominant eye has a strong inhibitory effect. A typical receptive field in the non-dominant eye has a large inhibitory surround and a "non-inhibitory" centre. The gratings that we used in our experiment, when binocularly presented, are bound to excite the inhibitory surround in the non-dominant eye's receptive field, and consequently suppress the response of the cortical cell, even though the dominant eye may be receiving the right stimulus. Thus these units would respond to the lines of a grating, when they are presented to the dominant eye alone, but not for a binocular presentation. If neurones such as these could be involved in a colour-specific adaptation, it would explain our observers reporting pinkish vertical and bluish horizontal aftereffects when one of the two eyes was occluded.

MacKay and MacKay, after measuring the time-course of the McCollough effect, questioned[10] the concept[1] of selective adaptation of single units sensitive to colour and orientation, and suggested, alternatively, cooperative synaptic and subsynaptic changes among an associative network of neurones. I suggest that these changes could occur at a binocular site too. The binocular units may not themselves be colour coded, but they are at least actively involved in the hypothetical neuronal changes. The claims that the McCollough effect is confined to monocular channels[1-5] or that it could be just retinal[10] are not tenable. It can even be proposed that all McCollough adaptation occurs in the visual cortex, in accord with the electrophysiological findings of cells with only concentric receptive fields before the striate cortex in the visual pathway[13].

My results are consistent with the extensive neurophysiological evidence for colour coded cells in the visual cortex[14-19]. Though only a few of these cells possess both colour and spatial specificities[16], it can be suggested that all the colour sensitive neurones, whether they have orientation specificity or not, are involved in structural changes with orientation-specific binocular neurones to yield long lasting aftereffects contingent on colour and orientation. This model is basically similar to the one proposed by Leppmann[20] The relative sparsity of cells with both specificities and the hypothetical neural changes justifies the requirement of a strong cellular response (binocular synergy) to bring out an aftereffect, in contrast to the easy interocular transfer[2] of pattern adaptation.

My observations are also the first psychophysical correlates of some of the stimulus properties of the various binocular neurones described with electrophysiological techniques, namely, the facilitated binocular response[6-9] and the subtle inhibitory input from an otherwise uninfluential eye[12]. It would be interesting to apply our experimental paradigm to other phenomena, such as aftereffects contingent on colour and movement, that do not show[21] interocular transfer.

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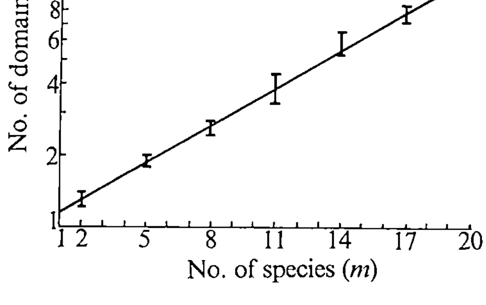
Multiple domains of attraction in competition communities

ECOLOGISTS typically explain the absence of a species in an area by searching for ultimate climatic or physical factors that limit its distribution or favour its competitors. The possibility that the system is not in equilibrium—that colonisation has not occurred—is not normally considered. Even less appreciated is that there may be a number of alternative stable equilibrium communities which may develop in a given area and that the final outcome may depend solely or partially on historical factors such as the sequence and numbers in which each species colonises. Here we investigate the theoretical possibility of this proposition for competition communities.

Examples of multiple domains of attraction are present in intertidal communities[1], microbial systems[2] between flour beetles[3] and on defaunated mangrove islands[4]. Diamond's study of competition among 400 bird species on the several hundred islands off the New Guinea archipelago offers some excellent examples[5]. He was forced to go beyond the normal competition theory and to introduce the concept of community assembly rules (forbidden and permitted subsets of species) and transition probabilities between them. We here investigate some of the dynamical underpinnings of species assembly rules.

Fig. 1 Number of domains of attraction against the initial number of species in the competition system. The mean interspecies competition ($\bar{a}$) is 1 0. Bars indicate ±1 s.e.



Unfortunately, it is impossible to analyse analytically such global stability problems as this for large numbers of interacting species. The local (or Liapunov) stability analyses of ecological communities of May[6], Gilpin[7], Roughgarden[8] and others is inappropriate here. We have opted to explore state-space landscape of interspecific competition communities by numerically integrating a normalised version of the Lotka[9] and Volterra[10] equations:

$$dN_i/dt = N_i(1 - a_{ij} N_j) \qquad (1)$$
$$i = 1 - m, \quad a_{ii} = 1, \quad a_{ij} \geq 0$$

where N_i is the population density of the *i*th species and a_{ij} is the competition of the *j*th species on the *i*th species. Each species has a low density exponential growth rate of 1 in the absence of other species and reaches a single species equilibrium of 1. For a random assignment of the a_i, we wish to know the number of domains of attraction of the system and their probability of occurrence. We could do this by testing the feasibility, stability and resistance to invasion of the 2^m different combinations of the *m* species. But this is very costly for large *m*, and, moreover, tells nothing about the probability of occurrence of the different domains of attraction. We have therefore chosen to randomly initialise the system on the hyperplane $\Sigma N_i = 1$. Thus, each initialisation corresponds to one possible combination of relative abundances of the potential colonising species pool. Equation (1) is then solved by computer iteration until a stable domain is reached. During the course of this iteration certain species will become extinct. It is theoretically possible that a limit cycle (or perhaps ergodic behaviour) would result, but in the several hundred systems we explored we did not find any such case. Perhaps this was due to the biologically realistic truncation used—any species that fell below one one hundred thousandth of its single species equilibrium went extinct.

We initially used colonising pools species (*m*) of size 2, 5, 8, 11, and 14. Values of *a* were drawn from a rectangular distribution with a mean interspecies competition coefficient ($\bar{a}$) of 0.35, 0.5, 0.65, 0.8 and 1.0. In one series the *a* matrix was symmetrical; in another all the terms of the *a* matrix were random; in a third series the *a* matrix was half a symmetrical matrix plus half a random matrix. For each *m* and $\bar{a}$ combination, 10 matrices were generated each of which was solved with 20 different initialisations. A multivariate analysis of the resulting data—that is, the number of domains of attraction compared with community size and mean strength of interspecies competition—produced a single parameter model that explained almost 90% of the experimental variance

$$D = \exp[p(m-1)\bar{a}] \qquad (2)$$

where *D* is the number of domains of attraction and *p* is an empirical parameter. For the symmetrical, mixed and random matrices, respectively, *p* has the values (±s.e.) 0.182±0.025, 0.084±0.014, and 0.062±0.011. Thus competition matrices which are symmetric, as might be the case for resource competition between species with equal niche breadths and single species equilibria, give more domains of attraction than nonsymmetric competition. The latter might occur where there is interference competition or where there is much dimensionality in niche packing.

To test empirical relation (2) for the functional relationship between *D* and *m*, we ran a more exhaustive series of tests in which each competition matrix was initialised 200 times; the *a* matrices were half symmetrical and half random and $\bar{a}$ was 1.0; *m* was varied between 2 and 20 in steps of 3. The results are shown in Fig. 1. The logarithmic relationship between *D* and *m* is confirmed, although the regression line misses the point ($D = 1$, $m = 1$), passage through which is logically necessary, by a few per cent. This could be corrected by adding a small constant to the right hand side of equation (2).

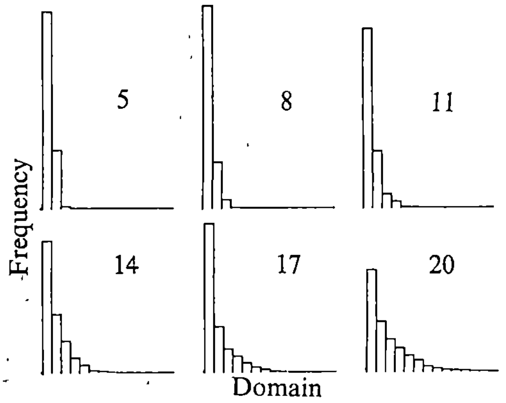


Fig. 2 Relative frequency of the domains of attraction for different initial community sizes. The domains are ordered by decreasing the frequency.

It is clear that relation (2) cannot hold for arbitrarily large values of $\bar{a}$, since for any *m* there exists an upper bound on the possible number of alternative equilibria. In the range of $\bar{a}$ that we investigated, however, the exponential dependence of *D* on $\bar{a}$ was a better fit than a linear dependence.

The relative 'volumes' of the domains of attraction were determined by counting the number of times a particular competitive assemblage occurred over the 200 times each *a* matrix was initialised. In Fig. 2 these are plotted with histograms. The bar on the far left represents the average frequency of the most common domain for the 10 different competition matrices, the next bar is the next most frequent domain and so on. As *m* increases the distribution spreads out but the relative volume of the largest domain of attraction decreases only slightly.

In Fig. 3 we have plotted both the mean number of species in a domain (weighted average over all domains, *a* matrices, and initialisations) and the mean total density of species in the domains as a function of the number of possible colonising species (*m*), for an average *a* of 1.0. These asymptote at ordinate of 2.5 species and 1.4 times the single-species equilibrium density, respectively, at an *m* of about 10.

A particular solution with a small domain of attraction may be easily perturbed by species increases or random perturbations to a solution with a broad basin of attraction, from which only larger perturbations will knock the system into other potential domains. It is interesting in this context that there is no regularity with the number of species present in a particular solution and the size of the domain of that solution for a particular *a* matrix. For example, stable domains containing both very few (for example, one) and large number of species, may be relatively unstable to global perturbations. Neither have we been able to discern any obvious heuristic rules in our trials which enable us to predict the attributes of the largest domains of attraction based on either species number,

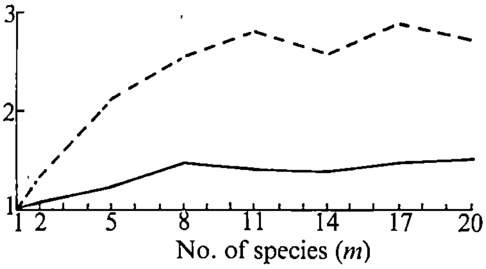


Fig. 3 Mean number of species present in a domain of attraction against initial number of species in the community (dashed line) and the mean total density ΣN_i of the species in a domain of attraction against the initial number of species in the community (solid line).

total density of all species, or combinations of these variables.

Since these results come from equations that are no more than a caricature of ecological reality, our results must be taken as provisional. Nonetheless, some interesting and practical conclusions may be drawn. Pest eradication programmes or the introduction of biological control agents may be viewed in some situations as global perturbations of community equilibria, or as attempts to go from one stable domain of attraction to another which is economically or aesthetically more desirable. The likelihood of success will depend on the relative size of the domain of the existing community compared with the size of the desired community, and the size and direction of the manipulated perturbation (for example, the number of individuals introduced). In some situations there may only be a few starting configurations of colonisation sequences leading to the formation of an economically desirable community. The lack of initial success in a species introduction plan does not necessarily mean that success will not be reached using another stocking rate or at a different time when resident species numbers are different.

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Seasonal effects on carbon isotope composition of cactus in a desert environment

IN plants with crassulacean acid metabolism (CAM), CO_2 is fixed in the light by ribulose-1,5-bisphosphate carboxylase[1,2] and in the dark by phosphoenol pyruvate (PEP) carboxylase[2,3]. Because these two enzymes fractionate the stable isotopes of carbon (^{12}C and ^{13}C) in the atmosphere and discriminate against ^{13}C to different extents[4], the wide range of values of carbon isotope discrimination ratios ($\delta^{13}C$) found in these plants[5] has been attributed to different contributions of dark and light fixation to carbon gain[6]. The proportion of total carbon fixed at night can be altered by environmental variables such as temperature[7], water status[8] and photoperiod[5]. Predictable changes in $\delta^{13}C$ values have been observed when these environmental conditions have been varied. With one exception, all such studies have been performed in artificial conditions. Because the $\delta^{13}C$ ratio of CAM plants has been used as an indicator of environment in palaeoecological investigations[9], we have examined the sensitivity of these $\delta^{13}C$ ratios to seasonal environmental influences in a natural system. Using *Opuntia acanthocarpa, O. basilaris* and *O. bigelovii*, we found that the carbon isotope composition of the plants did not change during the experiment, indicating the insensitivity of this measurement to short term seasonal influences in this environment.

The experimental site was at an altitude of 289 m in the Phillip L. Boyd Desert Research Centre at Palm Desert, California, and had a climate characterised by the temperature and rainfall pattern shown in Fig. 1. Tissue for each analysis was sampled by taking cork-borer cores transversely through the cactus cladodes. Each determination[10] was based on at least three cores and for *O. basilaris* and *O. bigelovii*, they were sectioned into cortex and pith, using the vascular strands as a guide for the division. Total titratable acidity and malic acid content[3] were determined in addition to the $\delta^{13}C$ ratio. Figure 1 shows that the extent of the diurnal change in titratable acidity responded to rainfall, particularly in the autumn. These results agree with those obtained previously with *O. basilaris* on the same site[11,12]. In the previous study, nocturnal stomatal opening occurred briefly after rain, facilitating dark CO_2

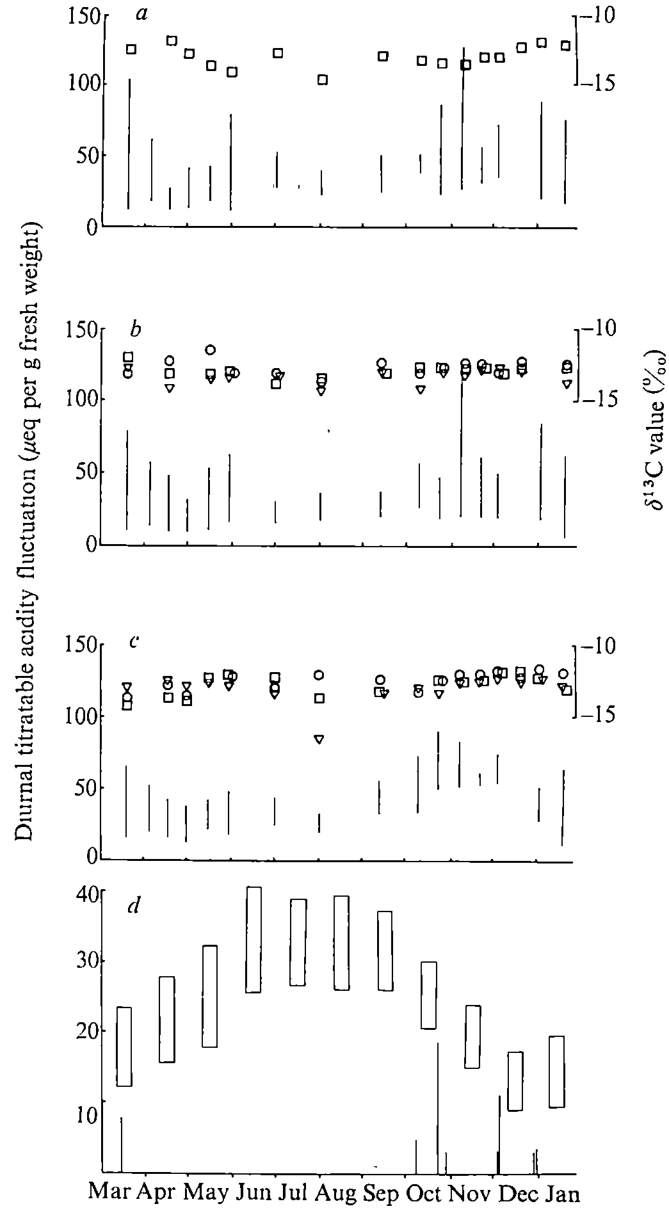

Fig. 1 Diurnal titratable acidity fluctuations (bars) and $\delta^{13}C$ values in natural populations of *O. acanthocarpa* (*a*) *O. basilaris* (*b*) and *O. bigelovii* (*c*) sampled in an environment with (*d*) the indicated mean monthly temperature ranges (blocks) and rainfall (bars). □, Bulk tissues; ▽, cortex; ○, pith. Titratable acidity was determined by titration of boiled aqueous tissue extracts to *p*H 7.0 with 0.01 N KOH. Correlation coefficients between $\delta^{13}C$ ratios and diurnal acidity changes: *a*, −0.0503; *b*, −0.3155; *c*, 0.1308; and time: *a*, −0.035; *b*, −0.209; *c*, −0.290. Mean annual $\delta^{13}C$ value and s.e.m.: *a*, −13.1±0.193; *b*, −13.0±0.085; *c*, −12.8±0.121.

fixation which resulted in increased diurnal fluctuations in acidity. At other times, stomata remained closed throughout the day and the small diurnal changes in acidity observed in these conditions were assumed to correspond to nightly fixation of respiratory CO_2 only. In no conditions did stomata open in the light This pattern of CO_2 uptake would involve only PEP carboxylase in the assimilation of atmospheric CO_2, and from this a stable $\delta^{13}C$ ratio of the magnitude observed in C_4 species would be predicted. Such a ratio was observed in this experiment (Fig. 1). Statistical analysis of the results indicated that the $\delta^{13}C$ ratios of *O. acanthocarpa* and *O. bigelovii* were correlated with neither the time of year nor the diurnal change in titratable acidity. The correlation between $\delta^{13}C$ value and diurnal fluctuation in titratable acidity for *O basilaris* was significant ($P=0\ 05$). But, the range of $\delta^{13}C$ values observed for all species was within the limits of observation and C_4 species[5]. In *O. basilaris* and *O. bigelovii* the $\delta^{13}C$ ratios measured in pith and cortex samples were not significantly different from the bulk value for the tissue. In other experiments (our unpublished data), although carbon fixed at night was initially found localised in the cortex, radial equilibration with the pith occurred in a few days.

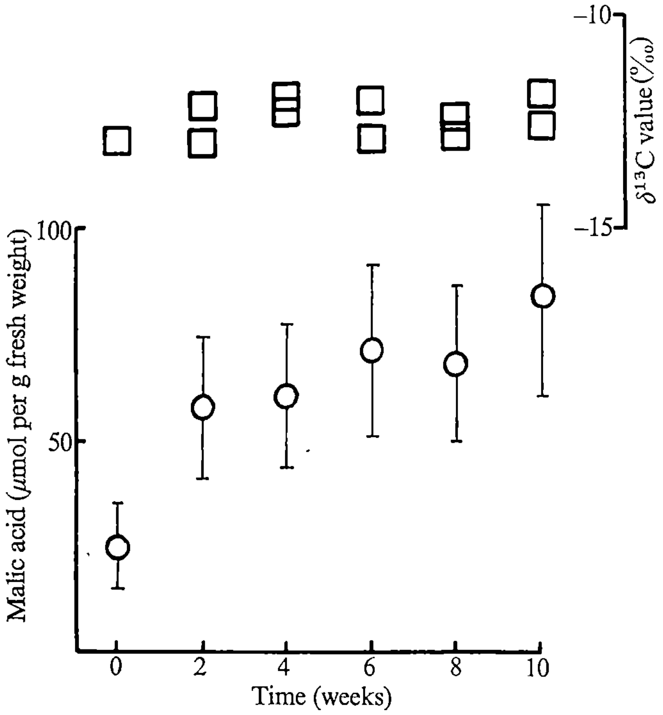

Fig. 2 Response of morning *O. bigelovii* malic acid levels and $\delta^{13}C$ values to irrigation in autumn A natural clone of plants was irrigated with 6–10 mm of water every 2 weeks. Correlation coefficient between $\delta^{13}C$ values and time was 0.412. Mean and standard error of the $\delta^{13}C$ values was $-12\ 6 \pm 0.137$. Vertical bars indicate 95% confidence limits for the malic acid estimation.

The sensitivity of the $\delta^{13}C$ ratio to environmental change was further examined in a clone of *O. bigelovii* which was subject to regular irrigation in autumn, when the cooler days and improved water status of the plants was expected to promote daytime CO_2 fixation[8], and therefore a decline in the $\delta^{13}C$ ratio. The results, presented in Fig. 2, indicate that although the production of malic acid at night increased markedly, the $\delta^{13}C$ ratio remained stable at a high value. The data presented here therefore indicate that the $\delta^{13}C$ ratio of the plants studied is relatively insensitive to short term seasonal changes in natural desert conditions. The data also confirm that no exogenous CO_2 is fixed in the light in this environment. These experiments imply then, that the varying $\delta^{13}C$ ratios observed in wild populations of cactus[1,9] are a result of long term environmental differences rather than seasonal effects. This result gives credence to the use of such ratios for the reconstruction of past climates[9].

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Real-time delayed tracking in flies

A DARK target on a bright background is fixated[1] and tracked[2,3] by a flying fly. The delay (dead time) in the fly's behavioural response to a change in the retinal image of the target is very small (about 20 ms)[3–5]. An artificial increase of this delay may give useful insight into the kind of control systems used by the tracking fly. Comparable psychophysical tracking experiments have been performed. For instance, when a televised display of a person's own behaviour in (closed-loop) pursuit tracking is delayed, his performance, as measured by time on target, is seriously impaired[6]. It is possible to delay the fly's response in artificial closed-loop conditions, and we have therefore used the effects of delayed visual feedback on tracking performance to test quantitatively a theoretical description of the visual orientation behaviour of flies[1,7,8].

A female fly (head fixed to the thorax) was attached to a fast torque meter, in the centre of a cylindrical panorama, as described before[1]. The torque signal is transduced into angular displacement of the 'panorama' through an analog simulation of the flight dynamics. In this way it is possible to simulate a free flight situation, in so far as the fly can track a target moving in the horizontal plane. In our experiments the moving target consisted of a black vertical stripe. The angular speed of the target was determined by a zero-mean Gaussian noise added to the torque signal of the fly. The torque, F, of the fly was delayed in steps of 78 ms by means of a standard tape recorder. The experimental situation is described by the following equation

$$[\theta\psi(t)+k\psi(t)]+k\omega(t) = -F(t-\varepsilon) \qquad (1)$$

where the angular error $\psi(t)$, represents the actual position of the target on the fly's retina. The terms in brackets on the left give the analog approximation of the flight dynamics (θ is the moment of inertia of the fly, k represents a rotational

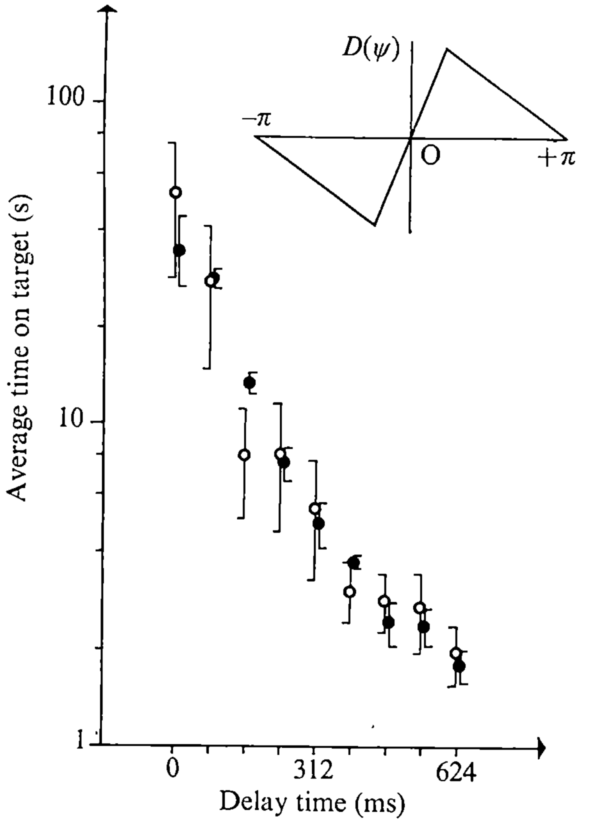

Fig. 1 Tracking performance under delayed response. The data points represent the mean time on target for six female flies, *Musca domestica* L. (○) and for four analog simulations of equation (1) (●). The vertical bars indicate the standard deviation of the mean. The inset shows the response function $D(\psi)$ which gives the ψ-dependent 'attractiveness' of a black stripe for the fly. $D(\psi)$ and the other parameter values used in the simulation are the same as those measured by Reichardt[1]. With these values, equation (2) without the terms $N(t)$ and $k\omega(t)$ shows oscillatory solutions for $\varepsilon \geqslant 156$ ms. Correspondingly, the associated linearised equation becomes unstable $\omega(t)$, the target angular velocity, is a Gaussian zero-mean process in all experiments, with a flat spectrum up to 15 Hz and an r.m.s. amplitude of about 50° s^{-1}. The width of the stripe was 2°, and its vertical length about 60°.

friction constant). $\omega(t)$ is the zero-mean Gaussian process representing the angular speed imposed on the target in our experiment. The average time on target T was measured as a function of the artificial delay ε. The object was considered to be lost by the fly when the angular error $\psi(t)$ between the direction of flight of the fly and the object reached ±170°.

In tracking behaviour, an increasing delay impairs performance: the greater the delay, the smaller is T (Fig. 1). The relationship between delay and time on target does not seem to fit a simple function. Psychophysical experiments, on the other hand, seem to show a simple inverse linear relationship between T (on a logarithmic scale) and magnitude of delay[6]. The orientation behaviour of flies and various tracking situations can be described quantitatively by a phenomenological theory[2–4,7–14], which leads to the equation

$$\theta\ddot{\psi}(t)+k\dot{\psi}(t)+k\omega(t) = -D[\psi(t)]-r\psi(t)+N(t) \qquad (2)$$

where the right hand side describes the instantaneous torque of the fly. $N(t)$ is a zero-mean Gaussian, band-limited random process, independent of visual input; $r\dot{\psi}$ represents a velocity-dependent, direction-sensitive optomotor response; $D(\psi)$ is a nonlinear anti-symmetrical function describing the fly's response to the stripe position (inset Fig. 1 and legend). All these terms have been characterised quantitatively[1,3,4,7].

If the right hand side is delayed by ε, equation (2) should represent our experimental situation. An approximate analytical solution in T has been obtained[9] in the case $\varepsilon = 0$ but seems otherwise extremely difficult. Therefore, to check the validity of the model, we carried out an analog simulation of equation (2), using the parameter values measured by Reichardt[1]. T was measured in the same conditions and with the same experimental arrangement as was used for the fly (Fig. 1). In the simulation we neglected the internal delay time of the fly (20 ms), and our results are consistent with previous evidence[3,7] that it can indeed be neglected.

The satisfactory agreement between the two sets of data of Fig. 1 supports the validity of the hypothesis underlying the phenomenological theory. Not only do the quantitative values of T as a function of ε agree within the limits of experimental error, but also the autocorrelations of the process $\psi(t)$ for the various values of ε are similar. In summary, equation (2), in addition to explaining various orientation situations quantitatively, also accounts for the delayed tracking described here. An important consequence is that the range of validity of the terms $D(\psi)$ and $r\psi$, representing the fly's visual induced response, extends through the rather extreme tracking conditions of our experiment. In particular, the function $D(\psi)$, which to a great extent determines the tracking performance, seems to be an almost invariant property of the visual control system of the fly.

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Determination of polarity in the amphibian limb

ACCORDING to the classical work of Harrison and his followers[1–6], the anteroposterior polarity of amphibian limbs consists of a 'molecular polarisation' of the cells which has been acquired by the late gastrula stage of development. I here present evidence that the polarity of the anteroposterior pattern is caused at a later stage of

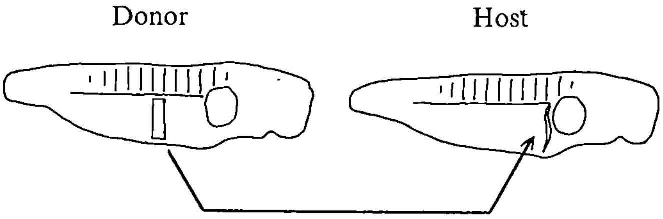

Fig. 1 Diagram of the reduplication-inducing graft described in the text. Operations were performed with electrolytically sharpened tungsten needles. The embryos were in half-strength Holtfreter solution containing 1/3,000 MS222 (Sandoz). The grafts were kept in place for 1–2 h with glass bridges. Embryos were kept in half-strength Holtfreter until the wounds had healed (usually overnight) and then transferred to 1/10 × Holtfreter containing 1/10,000 Nystatin (Squibb) and 1/2,000 Sulphadiazine (May and Baker). After hatching, larvae were kept in tap water which had been left to stand for 24 h. They were fed on brine shrimps (California Brine Shrimps Inc.) until all of the limb cartilages had developed, which takes about 4 weeks at 18 °C.

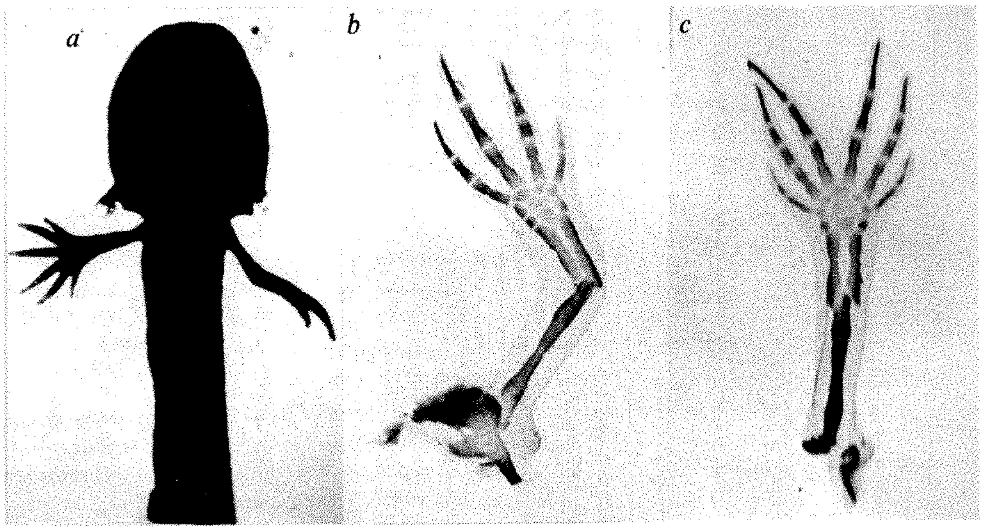

Fig 2 *a*, A reduplicated limb *in situ* showing its unusual posture; *b*, normal axolotl limb; *c*, reduplicated axolotl limb. For (*b*) and (*c*) the limbs were fixed in 5% trichloroacetic acid for 3 h, bleached overnight in Mayer's bleach, stained for 30 min with 0.1% Alcian green in acid acohol, differentiated overnight in acid alcohol, dehydrated and cleared in oil of wintergreen.

development by a signal emitted from a region posterior to the limb rudiment. In so far as this signal can travel in both directions it is not necessary to postulate any polarity at the cellular level.

The basic experiment which demonstrates the existence of the polarising region is shown in Fig. 1. It was performed on stage-34 embryos of the axolotl (*Ambystoma mexicanum*)[7]. A strip of tissue consisting of ectoderm and lateral plate mesoderm was grafted from a position ventral to the 6th somite into a slit made ventral to the junction of somites 2 and 3, that is, from the posterior to the anterior margin of the limb rudiment. In 15 out of 25 cases the host forelimb developed into a mirror symmetrical reduplication. These reduplications consist of two posterior halves arranged symmetrically around the mid-axis. Typically, they have five to seven digits and a corresponding number of more proximal elements (Fig. 2*b* and *c*). The pattern of outgrowth of the buds is also symmetrical. The direction of growth is lateral, rather than dorsoposterior, so that the reduplicated limbs stick straight out from the body when they are fully developed (Fig. 2*a*).

When the grafts were taken from an anterior position (ventral to the 3rd somite), the host limbs developed normally (6 of 6 cases), suggesting that the phenomenon is not a nonspecific wounding or grafting effect. When such anterior strips were grafted posterior to the limb rudiment (ventral to the 6th somite) the host limbs also developed normally (6 of 6 cases). So the cause of the reduplications cannot simply be a disharmonic combination of anterior and posterior tissues.

The main reason for thinking that the grafts of posterior origin emit some form of signal is that the reduplications are formed entirely from host tissues. There are several lines of evidence for this, but the most clear cut is that the effect can be produced by an interspecific graft from embryos of the Spanish salamander (*Pleurodeles waltlii*) to the axolotl, and the resulting reduplications consist entirely of axolotl-type elements (Fig. 3). The same may be shown by the use of vitally stained grafts. It therefore seems that the signal from the polarising region is not instructive. What it does in this experiment is to evoke the formation of normal host elements in an abnormal position.

The approximate extents of the polarising and competent tissues were determined by further grafting. Pieces of tissue from the flank were assayed for polarising activity by studying whether or not they produced reduplications when grafted anterior to the limb rudiment. Other strips of tissue were assayed for 'limb competence' by being grafted to the flank between the fore and hind limb rudiments. Because of the extensive regulative ability of this system such fragments can develop into complete supernumerary limbs if they are competent (Fig. 4). It is clear that the zone of limb competence lies ventral to somites 3–5 as described by Harrison[1]. But the polarising region extends well beyond this, the greatest activity lying in the dorsal lateral plate ventral to somites 5–8. Thus, although it is not possible to prove the existence of a sharp boundary by grafting experiments, there is certainly a considerable geographical separation between the two types of tissue.

It is now possible to explain the occurrence of redupli-

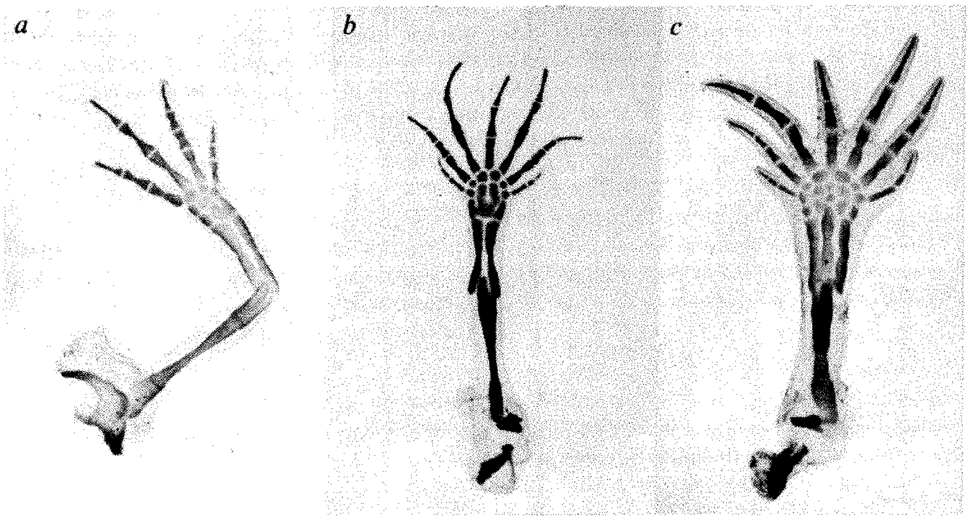

Fig. 3 *a*, Normal limb of *Pleurodeles waltlii*; note the spindle-shaped phalanges and the fusion of intermediale and ulnare in the proximal row of carpals. *b*, Reduplication produced by a pleurodele–pleurodele graft; all cartilage elements are of pleurodele character. *c*, Reduplication produced by a pleurodele–axolotl graft; all cartilage elements are of axolotl character.

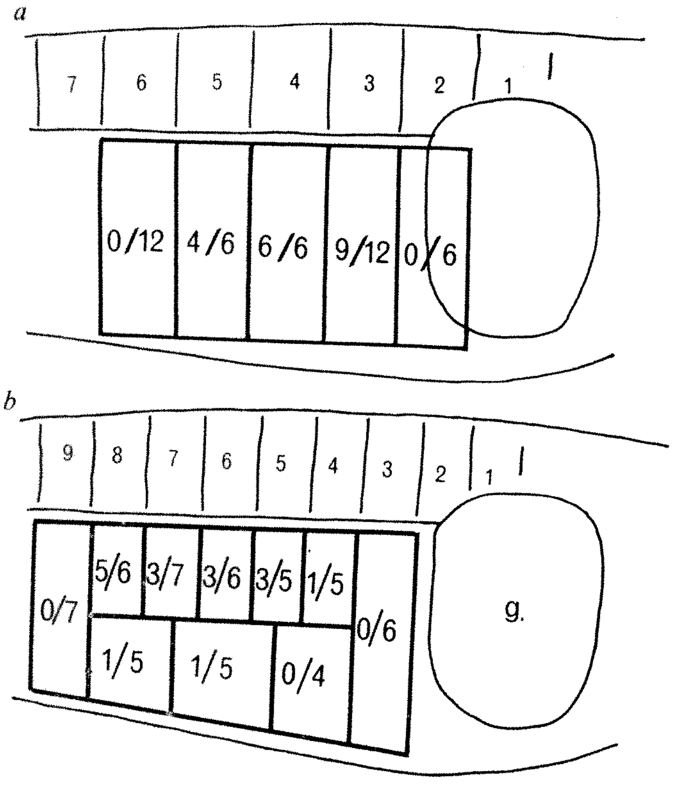

Fig. 4 *a*, Distribution of limb competence in stage-34 axolotl embryos, as assayed by the ability of fragments to form limb buds when grafted to the flank. *b*, Distribution of polarising ability at the same stage, as assayed by the ability of fragments to produce reduplication of the host forelimb when grafted ventral to the junction of somites 2 and 3. g, Gill rudiment. For both (*a*) and (*b*) the numbers in each box represent the number of positive cases over the total number of grafts for this particular fragment of tissue.

cations in the earlier, classical experiments. Most of that work involved rotations and transplantations of the so-called 'limb disks'. These consisted of lateral plate tissue extending from the level of the 6th somite to the anterior edge of the 3rd and therefore must have contained both polarising and competent tissue.

When such a limb disk was grafted to the normal site in normal orientation it gave a normal limb. When it was grafted to the normal site in reversed anteroposterior orientation it gave a reduplicated limb[2]. This is presumably because in the former case the polarising tissue remained in its usual posterior position, but in the latter case it was present on both sides of the competent tissue, as in the experiments described above. When a limb disk was grafted to the flank with reversed anteroposterior orientation, it gave a normal limb of reversed polarity. When the graft was normally orientated on the flank it often gave a reduplication[2]. This result was particularly mystifying to Harrison because the reduplications arose from the graft which was 'harmonic' with respect to the body axis. It is no longer surprising, however, because it can now be seen that it was the normally orientated grafts which had polarising tissue on both sides.

In addition, it is probably the polarising region that is responsible for the polarity of inducted limbs. Balinsky[8] and Takaya[5] showed that supernumerary limbs could be induced from the flanks of newts by implantation of fragments of nasal placode tissue. Nearly all these supernumeraries were single and of reversed anteroposterior polarity with respect to the body. This is just what would be expected if the placode induces a new zone of competent tissue posterior to the host polarising region.

Considering more recent work, it is clear that the existence of a posterior polarising region makes amphibian limb development seem much more like that of other vertebrates than previously thought. A polarising region has been described in the limb buds of the chick and mouse with very similar properties[9-12]. There remain two important differences, however: first, the interaction in *Ambystoma* and *Pleurodeles* seems to occur several days before the formation of a visible limb bud, whereas that of the chick is active during bud outgrowth; and second, the urodele limb rudiment is highly regulative in the anteroposterior axis whereas that of the chick is not.

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Langerhans cells form a reticuloepithelial trap for external contact antigens

THE outermost covering of the body, the epidermis, is a thin epithelial sheath comprising three distinct cell types, keratinocytes, melanocytes and Langerhans cells. The physicochemical and photo-protective role of the first two is well known, but the function of the third is still unknown, over a hundred years since its discovery[1]. The Langerhans cell was reported earlier as having a sensory role, or as being an effete form of the dendritic malanocyte below it[2], but it occurs in the dermis[3] and is mesenchymal, rather than neuroectodermal, in origin. Its fine structure reveals no melanin, but distinctive racquet-shaped granules. It has also been proposed that it is a macrophage[4] or that it may be involved in keratinisation, but recent evidence has suggested an immune function for the Langerhans cell in the development of contact dermatitis[5,6]. To investigate this possibility, we have exposed separated epidermal sheets to metals, aldehydes and amines known to act as contact antigens, and have observed their distribution histologically. We report here the selective uptake of antigens causing allergic contact dermatitis by Langerhans cells of the epidermis in guinea pigs and man.

Adult male white Hartley guinea pigs (300 g) were killed by cervical dislocation. After electric clipper removal of hair, ears were excised and inner and outer skin layers removed from cartilage by forceps. Human specimens were superficial horizontal skin slices removed by razor blade, with or without local lidocaine anaesthesia from male and female white adults with normal skin. The epidermis was finely dissected free after incubation in buffered EDTA solution for 2 h 37 °C (ref. 7), or in 1 M sodium bromide (pH 6.8, 0.1 M phosphate buffer) for 30 min. To brace metal antigens, the epidermal sheets were kept in mercuric chloride (7 mM), nickel chloride hexahydrate (12 mM), cobaltous chloride hexahydrate (12 mM), or chromic chloride hexahydrate (12 mM) in Tris-maleate buffer, pH

7.4 with 5% glucose for 60 min at 37 °C. The sheets were developed by immersion in 175 mM ammonium sulphide for 20 min at room temperature (22 °C), and then fixed in cold cacodylate formaldehyde (4 °C) for 20 min. This was followed by a 4-min exposure to Timm silver nitrate developer. Control staining of the Langerhans cells used lead nitrate (6 mM) in adenosine-5-triphosphate (ATP), glucose, Tris-maleate buffer with $MgSO_4$ added. The specimens were mounted in glycerine jelly.

The uptake of formaldehyde and glutaraldehyde was studied by exposing the separated epidermis to formaldehyde or glutaraldehyde (4%) solution for 20 min at 4 °C followed by paraphenylenediamine (PPD) (46 mM) in sodium borate–carbonate buffer (*p*H 10.8) for 60 min. Subsequent exposure to 3% hydrogen peroxide for 10 min at room temperature (22 °C) preceded mounting in glycerine jelly.

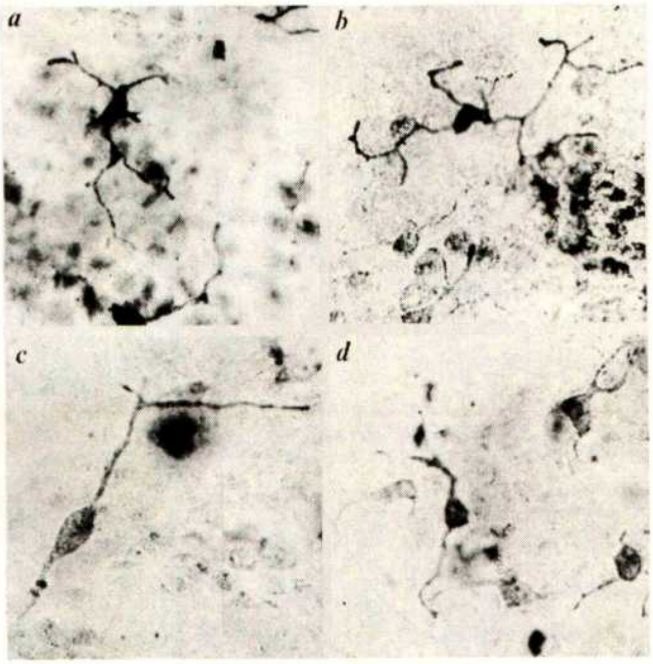

Fig. 1 Demonstration of selective uptake of various antigens by Langerhans cells in human epidermis. *a*, Mercuric chloride (ammonium sulphide developer, formaldehyde fix, Timm intensifier); *b*, nickel chloride (as in *a*); *c*, chromic chloride (as in *a*); *d*, formaldehyde (paraphenylenediamine developer, hydrogen peroxide intensifier). (×352.)

Amine uptake was studied by exposing fresh, unfixed epidermal razor biopsy specimens to PPD (46 mM) in borate buffer (*p*H 10.8), or to ethylenediamine dihydrochloride (8 mM) in 5% glucose in water for 20 min at 37 °C. The PPD-treated epidermis was then exposed to 5% *o*-phthaldialdehyde in absolute ethanol for 10 min at room temperature and mounted in xylene. The ethylenediamine-treated epidermis was exposed to 5% *o*-phthaldialdehyde in xylene for 10 min at room temperature and mounted in xylene.

All the antigens listed were selectively taken up by high level dendritic cells which could be identified in controls as Langerhans cells by the standard ATPase stain[7]. The metallic antigens, mercury, nickel and chromium (Fig. 1*a–c*) produced particularly clear outlines of the cell body with long dendrites coursing between the adjacent keratinocytes. Cobalt deposition was equally specific, although the dendrites here appeared shorter and less filamentous.

The uptake of formaldehyde clearly delineated the high level dendritic cell (Fig 1*d*), as did glutaraldehyde. The uptake of PPD and ethylenediamine by the Langerhans cells could be seen clearly only when fresh epidermal shavings were used, and it was difficult to achieve uniform staining. All the results were essentially the same in guinea pigs as in man.

This study reveals a hitherto unknown affinity of the Langerhans cell network for the most commonly known external contact sensitisers and indicates that haptens or antigens which penetrate the stratum corneum into the living avascular epidermis are trapped by a specialised protective reticulum of dendritic cells. We call this network of Langerhans cells a reticuloepithelial system, indicating its close relationship to the reticuloendothelial system which clears the body of antigens which have entered the general circulation. Thus, the epidermis has a trap for external contact antigens which parallels that of the lymph node and spleen for circulating antigenic material. The cells which remove foreign antigens from the epidermis are metallophilic, as are the specialised non-phagocytic reticuloendothelial cells of the spleen[8]. The Langerhans cells are further characterised by a high level of ATPase activity typical of reticuloendothelial cells[9]. Even more striking is the dendritic nature of the Langerhans cell, which duplicates that of the antigen-retaining cells of the lymph nodes and spleen[10]. These dendrites help the cell in its protective and clearing functions and in well stained specimens they form a virtually impassable intercellular system high in the epidermis.

The anatomy of the epidermis does not require classical phagocytes, since it is molecular, rather than particulate, matter which enters this syncytium. The Langerhans cell is thus a specialised mesenchymal scavenger which engulfs and processes chemicals entering the epidermal intercellular spaces from above or below. It must metabolise many compounds by its highly active intracellular enzyme system[4], but other compounds such as we have studied persist as sensitising antigens localised to the Langerhans cell. This cell seems to be the target cell for cytolysis and immunopathology in contact dermatitis and could be a new model for observations on the nature of delayed hypersensitivity. Our investigation was limited to antigens which could be identified by direct histological techniques, but it should be possible to trace other antigens to this reticuloepithelial system by appropriate radioactive and immunofluorescent techniques. In any event, the monoplanar topography of the Langerhans cell system within the upper epidermis may make it a more attractive test model for study than the analogous antigen-trapping cell system hidden within the spleen, lymph node and blood vessels.

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Ability of activated complement components to induce lysosomal enzyme release from macrophages

THE mechanism underlying chronic inflammation is of considerable medical importance both in temperate countries—where diseases such as rheumatoid arthritis are common—and in tropical countries—where various chronic inflammatory conditions cause much disease and disability. It is generally accepted that the macrophage is the central cell in chronic inflammation. We have drawn attention to the parallelism between the capacity of various agents to induce chronic inflammation *in vivo* and selective lysosomal enzyme secretion from cultures of macrophages[1,2]. The mechanism by which macrophage enzyme secretion is induced is unknown: we now report a mechanism mediated by cleavage products of the complement component C3.

Mononuclear phagocytic cells interact with complement in several ways. Human monocytes, mouse peritoneal macrophages and rabbit alveolar macrophages have receptors for C3b (refs 3 and 4). The experiments reported here show that attachment of C3b to mouse macrophages in culture results in a dose-dependent and time-dependent release of lysosomal enzymes into the medium. Figures 1 and 2 show data for *β*-galactosidase and *β*-glucuronidase, and similar observations have been made with *N*-acetyl-*β*-D-glucosaminidase. The selectivity of the release, which occurs without cell killing, is shown morphologically and by the failure of lactate dehydrogenase to appear in the medium; the levels of this enzyme increase in cells to which C3b is attached. The C3a fragment induces release of histamine from cells that store this substance[5]. We have observed that addition of C3a to macrophage cultures also results in release of acid hydrolases. But when the time course was studied, lysosomal enzyme release in the presence of C3a was always accompanied by loss of cellular lactate dehydrogenase (Figs 2 and 3), showing that the cells had been killed. The distinct effects of C3b and C3a on mononuclear phagocytes *in vitro* are interesting in view of the possible differences in the ways by which they interact with membranes.

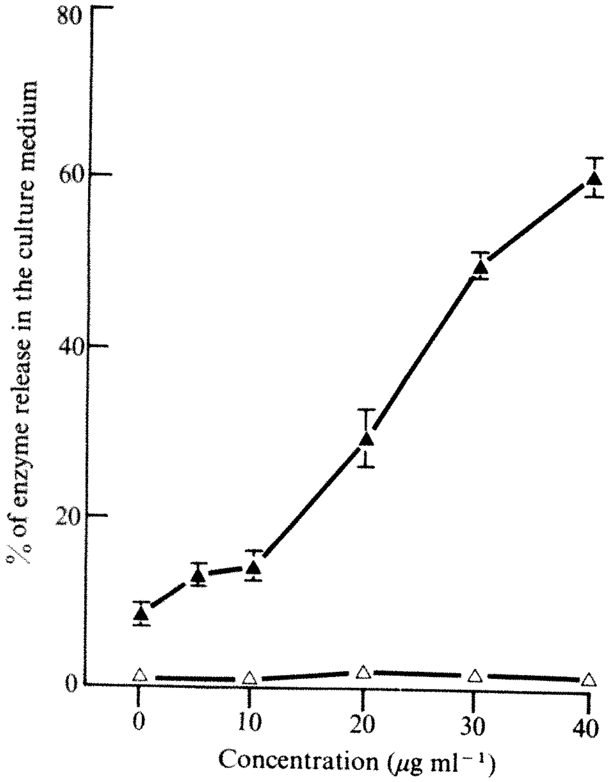

Fig. 1 The release of β-galactosidase (▲) and lactate dehydrogenase (△) from macrophages exposed to increasing concentrations of C3b. Mouse peritoneal macrophage cultures were prepared as before[12]. C3b was generated and purified as before[13]. Cells were exposed to increasing amounts of C3b for 72 h. At this time the culture medium was removed and the adherent cells were washed once with phosphate-buffered saline and then released by adding saline containing 0.1% (w/v) Triton X-100 (BDH). All fractions were assayed for β-galactosidase activity by the method of Conchie *et al.*[14]. Lactate dehydrogenase was assayed by determining the rate of oxidation of reduced nicotinamide adenine dinucleotide at 340 μm. Each value represents the mean ± s.d. of four separate plates.

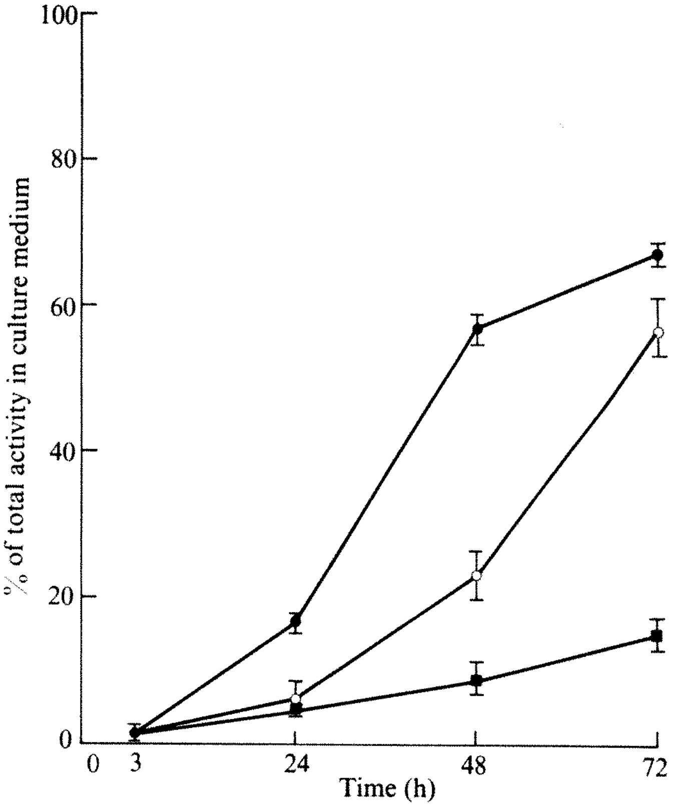

Fig. 2 The time dependence of macrophage β-glucuronidase release induced by the purified complement components C3 (■), C3b (○) and C3a (●). Highly purified guinea pig C3 was prepared as before[15]. For generation and purification of C3a, purified C3 was incubated with the C3-cleaving complex which is formed when cobra venom factor interacts with factor B, factor D and Mg^{2+} (ref. 16). This reaction mixture was put over a column of Sephadex G-100 (Pharmacia). The fractions which mediated contraction of isolated terminal guinea pig ileum were pooled and concentrated. Purified C3, C3b and C3a were provided by Dr D. Bitter-Suermann, Institut für Medizinisch Mikrobiologie, Mainz. The experimental conditions were identical to those described in Fig. 1. Each point represents the mean ± s.d. of four separate plates.

It is now clear that both granulocytes[6,7] and macrophages, phagocytic cells associated with acute and chronic inflammation respectively, can selectively release lysosomal hydrolases through the mediation of complement cleavage products, with no detectable loss of viability. This phenomenon may explain the ability of macrophages to cause tissue damage and degradation at sites of chronic inflammation while retaining their viability over long periods. This interpretation is supported by evidence that other agents inducing chronic inflammation, such as group A streptococcal cell walls[8], dental plaque[9], carrageenan[1,2] and mouldy hay dust (our unpublished results with J. Edwards), also cause selective release of lysosomal hydrolases from viable macrophages *in vitro*. All these agents

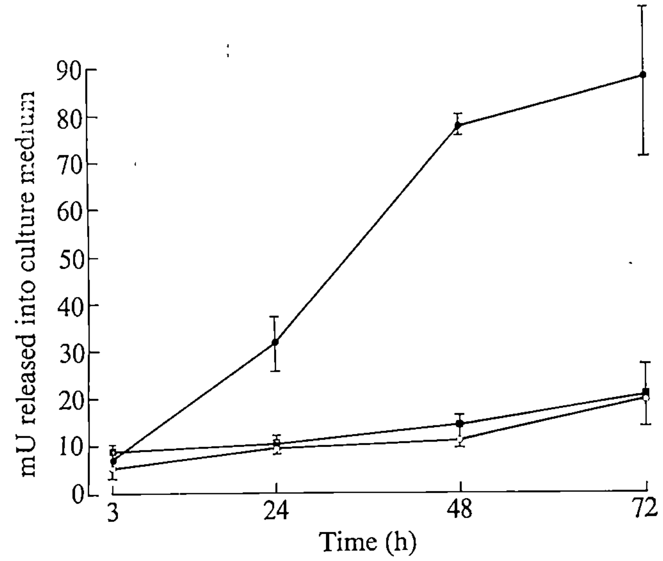

Fig. 3 Time-dependent release of lactate dehydrogenase from macrophages exposed to the purified complement components C3 (○), C3b (■) and C3a (●). The experimental conditions were identical to those described for Figs 1 and 2. Each point represents the mean ± s d. of four separate plates.

have been shown to activate complement by the alternative pathway[10,11], so generating C3b, which may be the common factor mediating enzyme release from macrophages. This system is amplified because we have found that enzymes released from stimulated macrophages can cleave C3, generating more C3b, which would, in turn, induce further enzyme release. The role of this amplification system in chronic inflammation clearly merits detailed study.

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Isologous IgG-induced tolerance to benzyl penicilloyl

INDUCTION of tolerance by haptens linked to non-immunogenic carriers[1–5] is used widely, not only to investigate the cellular mechanism of tolerance[6] but also as a new approach to the treatment of experimental immune disease[7]. A potentially therapeutic application of carrier-induced tolerance is the treatment of allergic diseases. In this respect, systemic hypersensitivity reactions to penicillin constitute a major cause of death due to anaphylaxis, and penicilloyl determinant is involved in approximately half of the cases[8–10]. We report here the suppression of reaginic antibody to the benzyl penicilloyl (BPO) determinant in unprimed as well as primed mice.

Benzyl penicilloyl was bound covalently in the penicilloyl configuration to several protein carriers, and residual unbound hapten was removed by gel filtration[11]. IgG1 (MOPC245) mouse gamma globulin (MGG1) was prepared as before[12]. The following hapten carrier conjugates were used: BPO_{17}–ovalbumin (OVA) as immunising antigen, BPO_{23}–MGG1 as the tolerogen and BPO_{37}–bovine gamma globulin (BGG) as challenge antigen in the 4-h passive cutaneous anaphylaxis assay in Sprague–Dawley rats for mouse IgE antibody[13].

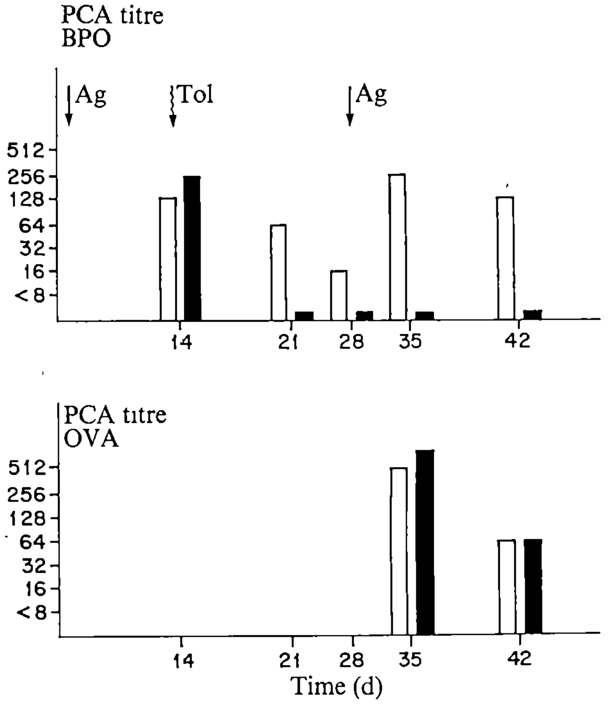

Fig. 1 Suppression of BPO IgE antibody in BDF_1 mice with BPO–MGG1 during an ongoing primary immune response. Each column represents the BPO and OVA PCA titre of pooled sera from either 10 control mice or 10 mice treated with tolerogen (Tol). The small arrows indicate the time of tolerogen or antigen administration. Open columns, control; black columns, treated with tolerogen.

The immunising regimen of Levine and Vas[14] was used to raise reaginic anti-hapten antibody in (C57BL/6×DBA/2)F_1 (BDF_1) mice. To determine whether the primary immune response to BPO could be suppressed by a single dose of tolerogen, a group of 10 (6–8-week-old) male BDF_1 mice were given 1 mg of BPO_{23}–MGG1 intravenously. Immediately thereafter these mice, as well as a group of 10 control BDF_1 mice, were injected intraperitoneally with 1 μg of BPO_{17}-OVA and 1 mg of $Al(OH)_3$ in 0.5 ml of phosphate-buffered saline (*p*H 7.4). The mice were bled on day 14 (which was shown in preliminary studies to correspond to the peak of the primary IgE antibody) and on days 21 and 28. After the third bleeding, both groups were again injected with BPO_{17}–OVA and alum and were bled 4 and 8 d later. The serum from individual mice was pooled to obtain sufficient for passive cutaneous anaphylaxis (PCA) titration. The titre of IgE anti-BPO antibody for the control group on day 14 was 1/128, 1/32 on day 21 and not detectable in serum diluted 1:8 on day 28. In contrast, in the group treated with tolerogen, no IgE antibody to BPO was detectable in serum obtained on days 14, 21 and 28 at a dilution of 1:8. The secondary response to BPO was also suppressed in the animals made tolerant to BPO–MGG1. The BPO PCA titre was 1/128 on day 4 and 1/2,048 on day 8 for the controls after the second immunogen injection. On both days, no BPO IgE antibody was detectable in serum diluted 1:8 in animals previously rendered tolerant.

To determine whether pre-existing IgE antibody response to BPO could be suppressed, two groups of 10 BDF$_1$ mice were immunised as described above. After 14 d both groups had IgE anti-BPO antibody in their serum (Fig. 1). At this time, one group of mice received 2.5 mg of BPO$_{23}$–MGG1 intravenously. All mice were bled 21 and 28 d after the initial immunisation. Both groups were subsequently injected with 1 μg of BPO$_{17}$–OVA in Al(OH)$_3$, and bled 1 and 2 weeks later. Figure 1 shows that the reaginic antibody to BPO was suppressed during both the primary and secondary response. In the secondary response, the suppression to BPO was hapten specific, since the response to the carrier OVA was unaffected.

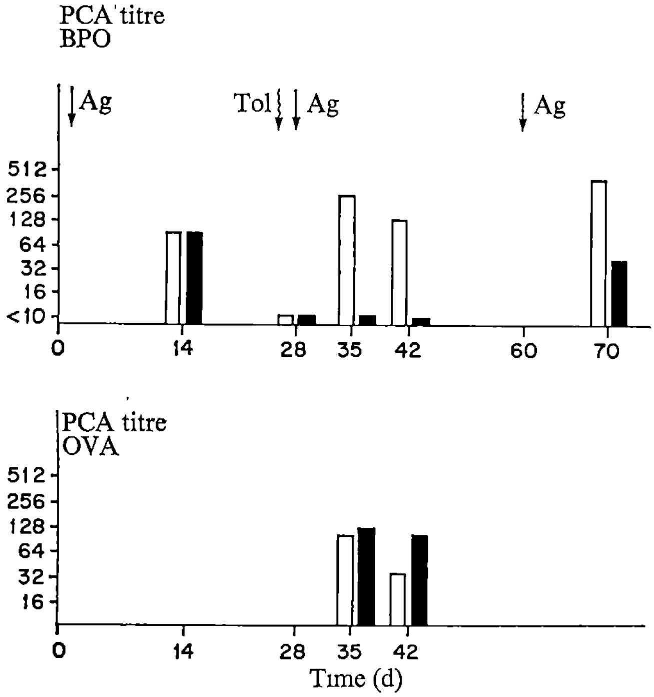

Fig. 2 Suppression of BPO IgE antibody in BDF$_1$ mice with BPO–MGG1 in the secondary immune response of primed mice. Each column represents the BPO and OVA PCA titre of pooled sera from either six control mice or six mice treated with tolerogen. The small arrows indicate the time of tolerogen and antigen administration. Columns as in Fig. 1

Finally, tolerance induction was studied in mice which had been primed with antigen after the IgE response had subsided. Immunised animals, given 1 mg of tolerogen before secondary immunisation with BPO$_{17}$–OVA, did not have detectable IgE anti-BPO antibody in their serum during the secondary response. In contrast, the IgE antibody response to the carrier OVA was the same in both groups (Fig. 2). After the third immunisation with antigen, the response to BPO was also diminished in animals treated with tolerogen before the second immunisation We believe we were dealing primarily with IgE Ab because the method of immunisation favours the production of IgE[14]. Mouse IgE but not IgG1 primed rat mast cells for PCA reaction[13] and finally the antibody involved in our experiment was susceptible to heating at 56 °C for 4 h (ref. 13).

The results suggest that the formation of reaginic antibody to the major determinant of penicillin can be suppressed in mice by injection of BPO isologous IgG before primary immunisation, during a continuing primary immune response, and before a secondary injection of hapten conjugate into primed animals. Suppression of the response was specific for the hapten, since IgE antibody to the carrier OVA was unaffected. The lack of fatal reactions in sensitised mice given 2.5 mg of BPO$_{23}$–MGG1 intravenously is interesting. The dose administered may constitute antigen excess in this system, and/or the presentation of the hapten on isologous Ig may have a protective effect. Katz and Benacerraf, using DNP–D–GL as a tolerogen[15,16], and Lee and Sehon, using DNP–MGG as a tolerogen[17,18], have shown that IgE antibody to DNP can be suppressed in either primed or unprimed rodents. Our studies extend these observations to an antigenic determinant which is prominently involved in human hypersensitivity reactions. The potential use of autologous human IgG as a carrier for induction of tolerance to antigenic determinants responsible for allergic diseases may have interesting implications

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Retransformation of a thermosensitive BALB/c–3T3 transformant by murine sarcoma virus at the non-permissive temperature

CERTAIN fibroblast cell lines exhibit some of the properties of transformed cells at one temperature while retaining normal properties at a higher temperature. For example, the spontaneous transformants of BALB/c–3T3 cells isolated by W Eckhart (unpublished results) can grow in soft agar at 32 °C (the permissive temperature) but not at 39 °C (the non-permissive temperature). They show transformed morphology at 32 °C but normal morphology at 39 °C (ref. 1). They have certain temperature-sensitive surface properties such as ability to grow in Agarose, loss of a transformation-sensitive iodinatable glycoprotein (LETS)[2,3], increase in total cell proteolytic activity[4] and loss of the growth promoting activity of fibroblast growth factor[5]. But they behave normally with respect to the regulation of intracellular events. Unlike transformed fibroblasts, both at permissive and non-permissive temperatures, BALB/c–3T3 cells exhibit density-dependent growth inhibition[5]. They cease growing as a result of serum limitation and express the typical increases in protein synthesis, ribosomal RNA synthesis and DNA synthesis when growth is initiated[1,6] The evidence therefore suggests that the transformants have lost their potential to express a transformed phenotype at the non-permissive temperature. I now show that this is not an irreversible loss by demonstrating that murine sarcoma virus (MSV) can transform the cells at the non-permissive temperature.

Table 1 Transformation assay of tsCl 1 and BALB/c–3T3 Cells with MSV at 39 °C

Virus dilution	No. of transformed foci per plate tsCl 1	No. of transformed foci per plate BALB/c–3T3	No. of labelled foci per plate tsCl 1	No. of labelled foci per plate BALB/c–3T3
10^{-2}	⩽500	⩾500	⩾500	⩾500
10^{-3}	150±20	87±12	185±24	105±15
10^{-4}	17±5	10±3	25±8	13±4
Mock infected	0	0	0	0

tsCl 1 or BALB/c–3T3 cells (10^6) were plated per 90-mm dish in Dulbecco's modified Eagle's medium containing 10% foetal calf serum (Flow). A day later, the cells were treated in 1 ml of medium containing polybrene (25 µg ml^{-1}) and Kirsten MSV (10^6 FFU) at the appropriate dilutions, or in mock-infected controls, for 2 h with frequent shaking. The cells were then allowed to incubate in medium containing 10% serum Transformed foci could be seen 3–4 d after exposure to virus and were scored at 7 d. Results obtained using a microscope, are expressed as the number of transformed foci per plate. Alternatively, on day 7, when the cultures became confluent, the cells were incubated in medium containing 1% serum and ^{3}H-thymidine (3 µCi ml^{-1}) at 1 µM for 24 h. Subsequent fixing and autoradiography of the dishes showed that only cell nuclei of transformed foci became labelled. Dishes were stained with Giemsa and results were expressed as the number of visible radioactively labelled colonies per dish All numbers represent averages of three separate determination ± two standard deviations.

An independent temperature-sensitive clone of BALB/c–3T3 cells (tsCl.1) was infected at 39 °C with a Kirsten strain of MSV (supplied by Dr G S. Martin). Transformed foci appeared in both tsCl.1 and the control BALB/c–3T3[7]-infected cultures. Both mutants and parent cell cultures were characterised with respect to saturation densities, regulation of DNA synthesis, growth in agar, and proteolytic activity in a casein overlay assay. The data presented here indicate that tsCl.1 can be transformed at 39 °C and that it can be rendered as fully transformed as the virally transformed cell lines Py3T3 (ref. 8) and SV3T3 (ref. 9).

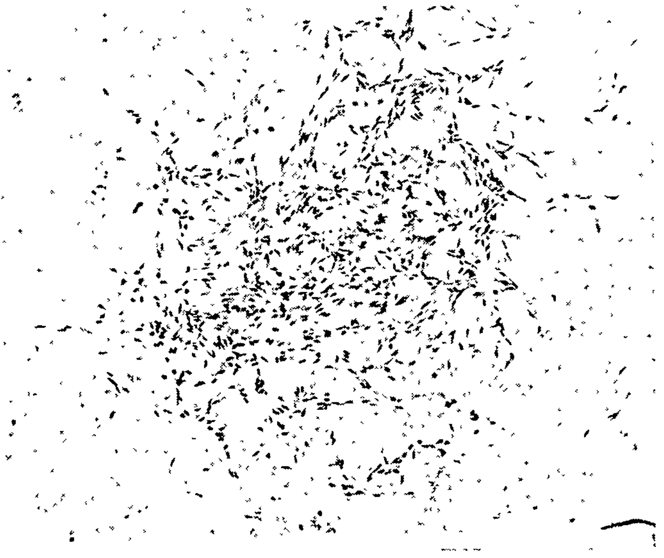

Fig. 1 Radioactively labelled focus of BALB/c–3T3 cells induced by MSV at 39 °C. Seven days after infection with MSV, the cells were exposed to ^{3}H-thymidine (3 µCi ml^{-1}) at 1 µM in Dulbecco's modified Eagle's medium containing 1% foetal calf serum for 24 h and processed as described previously. Cells were stained with Giemsa and photographed under the light microscope (×35).

Logarithmically growing cells were exposed to various dilutions of Kirsten MSV (10^6 FFU) at 39 °C. Foci were seen 3–4 d after infection but were routinely counted on day 7. As reported previously[10–12], their detection was difficult and required optimal culture conditions, such as rich medium and high inoculation densities. Transformed foci appeared as round refractile cells, adhered poorly to the plastic dish, had a tendency to float off into the medium and could not be stained easily. To screen large numbers of cultures for transformed foci rapidly, I developed a method which involves exposure of the virus-infected cultures to ^{3}H-thymidine in a medium containing low serum concentrations (0.5–1% foetal calf serum). In confluent cultures, transformed foci showed incorporation of ^{3}H-thymidine into labelled nuclei, whereas the uninfected parental BALB/c–3T3 cells remained unlabelled. As Fig. 1 shows, after autoradiography[13] and subsequent staining of the dishes, transformed foci could be recognised and counted. Table 1 shows the transformation assay of tsCl.1 and the parental

Table 2 Caseinolytic activity and growth in soft agar

Cell type	Temperature (° C)	Degree of casein hydrolysis (scale 0 to +4) MSV infected	Degree of casein hydrolysis (scale 0 to +4) Uninfected	Colony formation (%) MSV infected	Colony formation (%) Uninfected
tsCl.1	32	+4	+3	34	22
	39	+4	0	39	0.7
BALB/c–3T3	32	+3	0	20	0.3
	39	+3	0	28	0.5

Ten transformed foci were selected independently from each of the MSV-infected tsCl.1 and BALB/c–3T3 cells at various dilutions. While the uninfected parental cultures contained no detectable virus, all focus-derived lines showed evidence of virus production as determined by an XC plaque assay[11], thus excluding spontaneously transformed foci For the measurement of caseinolytic activity, untransformed BALB/c–3T3, mutant tsCl.1, and cloned MSV-transformed BALB/c–3T3 and tsCl 1 cells grown at their respective temperatures were each plated at 10^5 cells per 5-cm Petri dish in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum. After 5 h the monolayers were washed twice in serum-free medium and overlayered with 1 ml of medium at 45 °C, containing 0.6% Agarose (Difco), 2% powdered milk (Marvel), and 0.5% green African monkey serum (Gibco) The Agarose was allowed to harden at room temperature and the plates were then returned to incubate at 32 °C and 39 °C. The extent of plasmin-mediated caseinolysis was estimated after 40–50 h at 39 °C and after 80–120 h at 32 °C, based on a scale of 0 (no clearing) to +4 (complete clearing), according to the proportion of casein removed The ability of the cells described above to grow in soft agar was determined by plating 10^5 cells in medium containing 10% foetal calf serum in 5-cm Petri dishes. A day later cells were overlayered with 5 ml of a suspension of 0.33% agar (Difco) and 10% foetal calf serum in medium at 45 °C Cells were incubated at 32 °C or 39 °C and the number of colonies per dish determined 2 and 3 weeks later. The percentage of cells which grew in agar is shown Results represent averages of the focus-derived lines as determined in three separate experiments

BALB/c–3T3 cells at 39 °C. Transformed foci in the unstained dishes were scored, with the aid of a microscope, 7 d after infection. They were then compared with the numbers of labelled foci in the autoradiographed and stained dishes. The use of labelled nuclei makes possible detection of small colonies so that the numbers of labelled foci were slightly higher. The results showed essentially a one-hit response curve[12].

Fibroblasts transformed by DNA or RNA tumour viruses show markedly higher levels of proteolytic activity than do their normal untransformed counterparts[14,15]. This arginine-specific protease converts serum plasminogen to its enzymatically active form, plasmin. The plasmin in turn can be detected by its ability to hydrolyse milk protein in a casein overlay assay[16]. MSV-transformed tsCl.1 and BALB/c–3T3 foci were selected independently and grown at their respective temperatures. Monolayers of the cloned cultures and their normal counterparts were overlayered with Agarose containing casein in the presence of 0.5% monkey serum, and the extent of caseinolysis was measured at 32 °C and 39 °C. Thus, the transformed tsCl.1 and BALB/c–3T3 cells showed a marked caseinolytic activity over the non-transformed counterparts at 39 °C (Table 2). Similarly, when the cultures were trypsinised and tested for their ability to grow in soft agar[17], another parameter for measuring the degree of transformation in cell lines, the MSV-transformed variant and parent cells formed 39% and 28% colonies at 39 °C, respectively, compared with 0.7% and 0.5% colony formation of the uninfected cultures (Table 2).

Cloning efficiency and saturation density of the MSV transformants were higher at both temperatures compared with the uninfected cell counterpart at 32 °C. Thus, the final cell density of tsCl.1 in medium containing low serum concentration (1% foetal calf serum) was approximately twice as great at 32 °C than at 39 °C (2×10^6 and 0.9×10^6 cells per 5-cm dish at 32 °C and 39 °C, respectively), similar to untransformed 3T3 cells. In contrast, approximately a tenfold increase in saturation density of the MSV-transformed tsCl.1 and BALB/c–3T3 cells was observed over the normal phenotype. At low serum concentrations, MSV-transformed tsCl.1 cells reached saturation densities of 19×10^6 cells per 5-cm dish at 39 °C and 32 °C, respectively. Similarly, MSV-infected tsCl.1 cultures left to become confluent and exposed to ^{3}H-thymidine for 24 h at 39 °C and for 48 h at 32 °C showed incorporation of radioactivity only into nuclei of transformed foci. This was also the case for the parental BALB/c–3T3 cells (Fig. 1), which is another method of demonstrating that the MSV-transformed cells have overcome density-dependent inhibition of growth with respect to DNA synthesis.

Whether retransformation of a temperature-sensitive cell mutant at the non-permissive temperature is a general phenomenon that can be carried out with either DNA or RNA viruses remains to be determined. Renger retransformed temperature-sensitive SV3T3 cells with MSV at the non-permissive temperature[18] but the same cells could not be retransformed with high multiplicity SV40 (ref. 19). Similarly, SV3T3 revertants initially isolated by Pollack *et al.*[20], and the revertants of polyoma-transformed BHK cells[21] could not be retransformed by their respective viruses but could be rendered fully transformed by exposure to murine[18] or hamster sarcoma[21] viruses, respectively. In this study, however, tsCl.1 and another similar independent clone tsCl.10 (supplied by Dr W. Eckhart) could be retransformed both by an RNA virus (MSV) and by a DNA virus (SV40) at the non-permissive temperature. Unlike SV3T3, the SV40-transformed ts clones could grow in soft agar (not shown).

In summary, MSV-induced transformation of temperature-sensitive cell variants can be accomplished at the non-permissive temperature. Thus, I have demonstrated growth in soft agar, increase in caseinolytic activity, higher saturation densities and loss of regulation of growth and DNA synthesis after MSV transformation. My results suggest that a virus function can render these temperature-sensitive cells fully transformed with respect to certain properties of their intracellular machinery. Alternatively, the process of virus infection may induce a cell regulatory function. This possibility is being investigated, using a temperature-sensitive mutant of MSV to determine whether complementation can occur between the cellular mutation and the viral mutations which render the viral transformation temperature sensitive.

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Exceptional mutagenicity of a benzo[*a*]pyrene diol epoxide in cultured mammalian cells

BENZO[*a*]PYRENE (BP) is one of a group of chemically inert carcinogens which, in order to exert their cytotoxic, mutagenic and carcinogenic effects, require metabolic activation by mixed function oxygenases (MFO) to products capable of covalent reaction with cellular macromolecules[1–3]. More specifically, it has been shown in mouse skin[4] and various cultured mammalian cells[5], that, in the case of DNA, the extent of this reaction correlates with the carcinogenic potency of the compound. The hypothesis that DNA is the important target for this series of hydrocarbons is further supported by evidence that the most carcinogenic members are also the most mutagenic towards hamster cells *in vitro*[1]. Here we present evidence that a diol epoxide derivative of BP is a powerful mutagen when applied to mammalian cells, and suggest therefore that this compound is the ultimate carcinogenic metabolite of BP.

Although various models have been proposed regarding the nature of the BP–DNA reaction[6], convincing experimental evidence has been lacking. Recent chromatographic analysis of DNA from hamster embryo cells exposed to ^{3}H-BP, however, has established that, for this hydrocarbon, the binding species is a *trans*-7,8-dihydro-7,8-dihydroxy-9,10-epoxy derivative produced by a two-step metabolic event through the 7,8-epoxide[7]. Hulbert has predicted[8], on theoretical grounds, that two stereoisomers of this diol epoxide are possible (designated syn and anti, see Fig. 1) and that only one of these, syn, would be expected to react significantly with DNA *in vivo*. Both isomers have been synthesised and the stereochemistry of each confirmed by analysis of their integrated proton nuclear magnetic resonance (NMR) spectra[9].

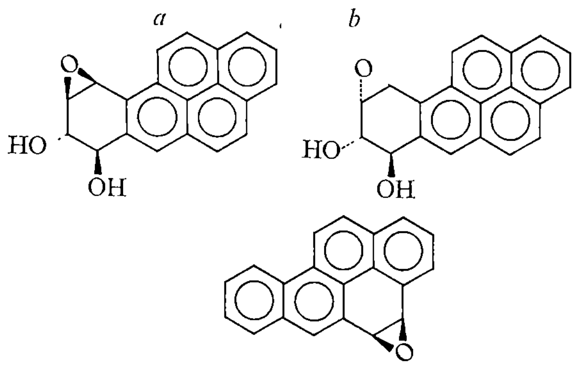

Fig. 1 Structural formulae of the three benzo[*a*]pyrene (BP) derivatives under investigation. *a*, BP diol epoxide syn configuration, *b*, BP diol epoxide anti configuration; *c*, BP-4,5-oxide (K-region epoxide). The two stereoisomers of BP diol epoxide have been designated syn and anti[9] to indicate the relationship of the epoxy group *cis* or *trans* to the 7-hydroxyl function. Hulbert[8] predicted that hydrogen bonding between these two groups, which is possible only in the case of syn, will facilitate opening of the epoxide, in effect producing an aralkyl carbonium ion on C9 or C10 of the molecule. This isomer would therefore react with appreciable S_N1 character and so show little preference *in vivo* for strong nucleophiles over relatively weak nucleophiles such as DNA. Anti and BP-4,5-oxide on the other hand, would be expected to react predominantly by an S_N2 mechanism and be much less reactive towards DNA, especially in the presence of strong competing nucleophiles such as the SH groups of glutathione and proteins.

We therefore thought it prudent to examine, in cultured mammalian cells, the cytotoxic and mutagenic properties of both diol epoxide isomers and to compare their respective activities with BP-4,5-oxide, the so-called K-region epoxide. For this purpose we used V79 cells, an established line with undetectable MFO activity[10] and, as a mutational marker, resistance to the purine analogue 8-azaguanine. This system is used widely to study mutagens which act directly and it is generally accepted that variants arise as a consequence of gene mutation. Our results indicate that all three compounds differ in their ability to induce mutations at this locus and that one diol epoxide isomer has exceptional mutagenic properties.

Figure 2 summarises the results of several experiments in which the three derivatives were compared in identical conditions. Parallel cultures of exponentially growing cells were exposed for 24 h to a range of concentrations of each compound, and replicate samples were plated for determination of induced cytotoxicity and mutagenesis (details in Fig. 2 legend). For each derivative a similar type of multitarget dose–survival curve was obtained, with a shouldered region at low doses followed by a steep exponential portion. The dose range required to achieve this response, however, varied, approximately in the ratio 1:3:6 for anti, syn and BP-4,5-oxide respectively. Similarly, the corresponding D_0 values were calculated as 0.10, 0.25, 0.85 μg cm^{-3} of culture medium. Further interpretation of the survival data in terms of the extent of the hydrocarbon–DNA reaction was precluded because labelled BP diol epoxides were not available.

The mutational response, as a measure of specific DNA modification, not only reflected the overall variations observed in the survival experiments but also revealed more subtle

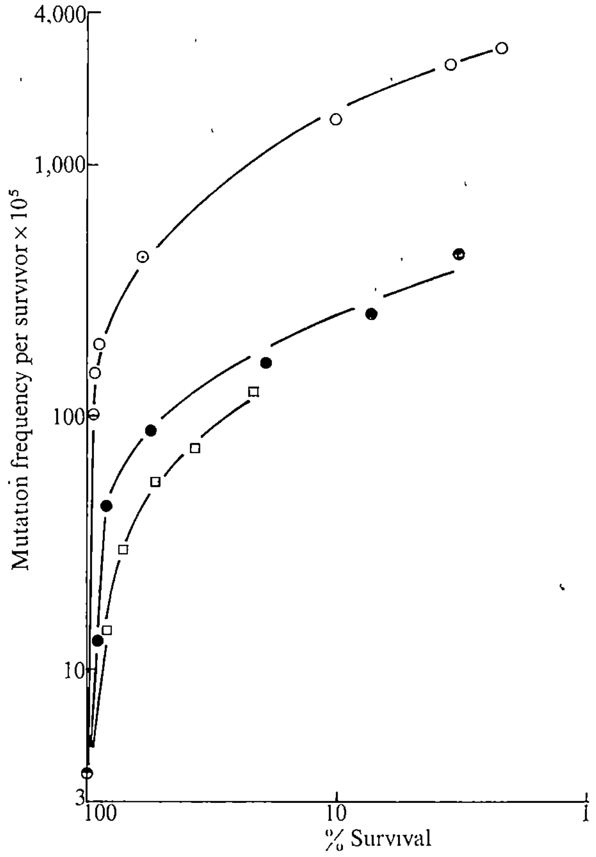

Fig. 2 Mutagenesis of V79 Chinese hamster cells as a function of percentage survival: ○, BP diol epoxide anti; ●, BP diol epoxide syn; □, BP-4,5-oxide. The results, derived from individual dose–response experiments, were expressed in this way to facilitate a direct comparison between the derivatives in terms of mutagenic efficiency. Mutation frequency was plotted on a logarithmic scale so that all three responses could be represented accurately on one graph. Linear–logarithmic plots of mutation frequency against survival respectively were linear below 85% survival. In all experiments 75-cm^2 plastic tissue culture flasks (Falcon), containing 30 cm^3 of Dulbecco's modified minimal essential medium with 10% foetal calf serum (Gibco), were each seeded with 8×10^5 cells grown from a clonal stock frozen in liquid nitrogen. After 18 h the resulting exponentially growing cultures were treated with various doses of the compounds added in 0.1 cm^3 of dimethylsulphoxide (DMSO). All hydrocarbon solutions were made up immediately before use in DMSO which had been dried over a molecular sieve. The precise concentration of each derivative in solution was established by ultraviolet spectrophotometry. After 24 h of treatment at 37 °C the cells were detached from each flask with trypsin–EDTA and the density of the resulting single-cell suspension was estimated with a haemocytometer. For the determination of induced toxicity, 2×10^2 cells, and for mutagenesis 5×10^4 cells, were plated in 5-cm and 9-cm plastic tissue culture dishes (Sterilin) respectively, and incubated at 37 °C in a humidified atmosphere of 5% CO_2:95% air. Mass selection of mutants defective in the purine salvage enzyme, hypoxanthine guanine phosphoribosyl transferase was effected by adding 8-azaguanine to the plates such that its final concentration in the medium was 30 μg cm^{-3}. To achieve maximum recovery of mutants, a complete expression time curve[11] was constructed for each dose of hydrocarbon. In practice, five to ten replicates were treated with 8-azaguanine at each of six expression time points ranging from 0 to 120 h after plating. Survival plates were stained after 7 d of incubation, mutation plates 3 d later, and colonies of more than about 50 cells were scored macroscopically. Mutation frequencies were corrected for toxicity and calculated per surviving cell. The values shown in the figure are those obtained from the peak expression times which ranged from about 48 h for shoulder doses to 96 h for the more lethal treatments. Control frequencies never exceeded 4×10^{-5} per survivor. We have already discussed the various pitfalls encountered in this type of experiment[12].

differences between the compounds. The dose response was cumulative for each compound, a characteristic observed by others who used these cells as targets for the classical mutagens EMS, MMS, X rays and ultraviolet light[13,14]. Although syn and BP-4,5-oxide proved relatively powerful mutagens, the absolute mutation frequencies obtained with anti were unexpectedly high. Wood *et al.* have tested BP-4,5-oxide in V79 cells[15] and the close agreement of our results with those for that epoxide served as a useful indication of the reproducibility of the system.

A double logarithmic plot of mutation frequency against survival is used frequently to discriminate between mutagens where the only measure of effective dose is induced toxicity. When the results discussed above are plotted in this way (Fig. 2) the true mutagenic potency of anti becomes clear, it being more than eight times as active as BP-4,5-oxide at 37% survival (e^{-1} = one lethal hit). This distinction between cytotoxic events and mutagenesis is best illustrated by applying the term "mutagenic efficiency"[16] to our data, which we define as the mean rate of increase of mutation frequency per lethal hit.

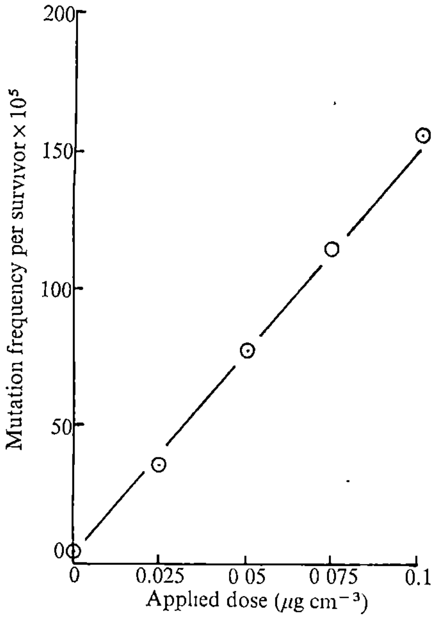

Fig. 3 The mutagenic activity of antidiol epoxide at shoulder doses. Experiments were carried out as described in legend to Fig. 2.

For all three compounds linear-logarithmic plots of induced mutation frequency against survival respectively were approximately linear when only the exponential portion of each survival curve was considered, thus reflecting a direct proportionality between the two effects. The response in each case can therefore be represented adequately by an equation of the form: $y = ax+b$, where a is the slope and thus the mutagenic efficiency, and b is the intercept on the y (mutation frequency) axis, resulting from a higher rate of mutagenesis at minimally lethal and subtoxic doses. For anti, syn and BP-4,5-oxide these equations were calculated as: $y_{\mathrm{anti}} = 10^{-4}\ (56x+20)$, $y_{\mathrm{syn}} = 10^{-4}\ (8.6x+2.3)$ and $y_{\mathrm{k}} = 10^{-4}\ (8.6x+0.1)$, respectively.

Although BP-4,5-oxide was, not unexpectedly, the least powerful of the BP derivatives tested, the observed differences between the two diol epoxide isomers are difficult to explain on the basis of Hulbert's arguments[8]. Indeed, the syn isomer was predicted to be the most active. It should be appreciated, however, that mutagenesis has been examined at only a single genetic locus so far, and this may not be representative of the whole genome or indeed those targets relevant to carcinogenesis. Also, we have not finally established which isomer binds to DNA after BP is metabolised *in vivo*. Nevertheless, we feel that the possible contribution of these findings towards the understanding of BP carcinogenesis cannot be ignored.

Further examination of the anti isomer with respect to its mutagenic potency at sublethal shoulder doses proved equally exciting. The results (Fig. 3) demonstrate that in the absence of measureable toxicity, mutagenesis increases proportionally with dose. The type of low dose experiment is likely to be much more representative of human exposure to environmental BP. Moreover, the induction of cancer through somatic mutation(s) may well require that a powerful carcinogen be an efficient mutagen, that is that it can induce the necessary mutations in the DNA of the target cell with the minimum of concomitant side effects. The anti isomer of diol epoxide discussed here certainly meets this requirement.

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Tumorigenicity and the expression of cell-surface carbohydrates

TUMOUR cell populations are heterogeneous with respect to various biological properties[1–3]. Immunological properties that influence tumour–host relationships and metastatic properties of tumours may also differ between subpopulations of tumour cells[4,5]. In addition, conditions of growth may favour the emergence of subpopulations, and the immunogenic modification of susceptible subpopulations. We have reported that highly immunogenic tumour cells can be isolated from the parental tumour by concanavalin A (con A)–affinity chromatography[6,7], and the relative proportion of different subpopulations varied with different conditions of growth. We now report that the tumorigenicity of murine adenocarcinoma varies as a function of the tumour subpopulations present, as defined by their expression of cell-surface carbohydrates.

The transplantable mammary adenocarcinoma A-10 (syngeneic to strain A/J mice) has been maintained as an ascitic tumour in B6AF$_1$ (C57BL/6×A/J) mice, and in monolayer culture using RPMI 1640 medium, supplemented with 10% foetal calf serum and antibiotics.

The tumorigenicity of A-10 cells grown *in vivo* and *in vitro* is summarised in Table 1. An intraperitoneal inoculum of 10^2 or 10^3 cells grown *in vivo* routinely caused 100% mortality of B6AF$_1$ mice, whereas similar inocula of cells grown *in vitro* caused about 50% mortality. Inocula of up to 10^6 cultured cells were not always 100% fatal (J.J.K. and

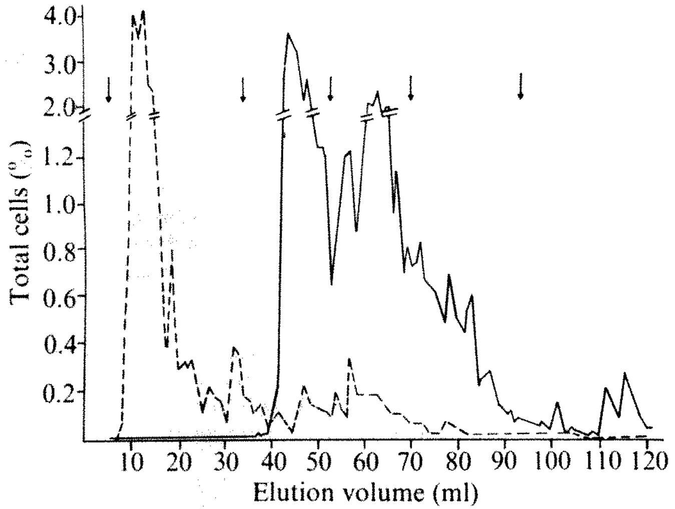

Fig. 1 The elution profile of A-10 cells from con A–affinity columns. The percentage of total cells is shown as a function of elution volume. This technique has been described in detail elsewhere[7]. Cells were prepared as described in Table 1, and eluted with the sequential addition (arrows) of PBS, α-methyl-D-mannoside, α-methyl-D-glucoside, sucrose and α-D-glucose. ---, Cells grown *in vivo*; —, cells grown *in vitro*. Ammonium oxalate wash of cultured cells did not affect the elution profile.

W.A.S., unpublished results). This difference in tumorigenicity was probably mediated by immunological mechanisms since an inoculum of 10^2 cultured cells killed 100% of immunosuppressed mice with an average survival time similar to that for mice inoculated with cells grown *in vivo*.

Cultured A-10 cells regained the tumorigenicity observed for cells grown *in vivo* when subsequently passaged as an ascitic tumour. Thus the tumorigenicity of this murine adenocarcinoma depended on conditions of tumour cell growth.

The elution profile of A-10 cells from con A–affinity columns also depended on conditions of cell growth (Fig. 1). Tumours grown *in vivo* contained a large subpopulation of cells eluted with PBS (not initially binding the column) and few cells were released by subsequent addition of sugar competitors for con A binding. In contrast, cultured A-10 tumour contained virtually no PBS-eluted subpopulation, and was characterised by subpopulations released with the different sugar solutions.

The elution profile of cultured A-10 cells was evaluated after the first and second passage in normal mice. As Fig. 2 shows, the first passage of cultured cells *in vivo* gave subpopulations characteristic of cells grown both *in vivo* and *in vitro*, that is, cells eluted with PBS, and large numbers of cells released by sugar solutions. The tumorigenicity of these cells was comparable with that observed for cultured cells (Table 1). But, after the second passage of cultured cells *in vivo*, the elution profile had reverted almost completely to that of tumour grown *in vivo*. A large subpopulation was defined by elution with PBS, and few cells were released from the column by sugar. This profile correlated with a marked increase in tumorigenicity.

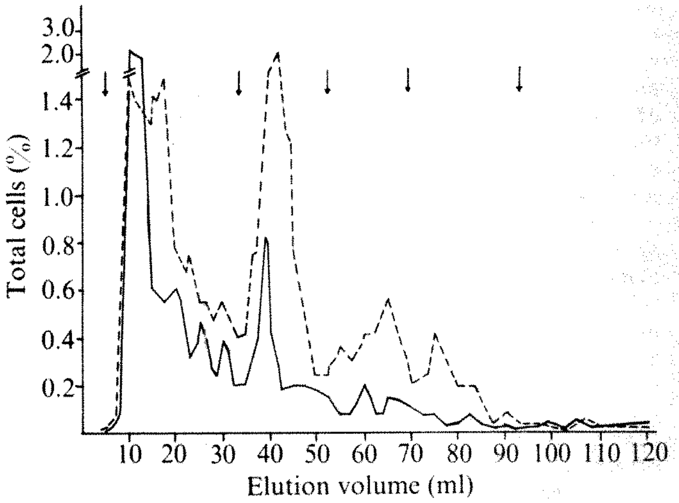

Fig. 2 The elution profile of cultured A-10 tumour after the first (- - -) and second (—) passage in B6AF$_1$ mice. The percentage of total cells is shown as a function of elution volume (see Fig. 1).

The antigenic properties of tumour cells and the expression of tumour-associated antigens have been altered by conditions of growth before[8–11], but few studies have correlated surface properties of intact cells with either progressive tumour growth or tumour rejection. Studies on the tumorigenicity and strain-specificity of another murine mammary tumour, TA3 (refs 12–15), have shown the presence of a membrane glycoprotein, epiglycan, which correlated with loss of strain-specificity of TA3–Ha cells. Epiglycan was lost reversibly after these cells were cultured, and correlated with a return of strain-specific tumorigenicity. When TA3–Ha cells were grown *in vitro* other membrane changes occurred[15], including a marked increase in the molar proportion of mannose to total hexosamine. In the light of our results, the relative increase of this con A-binding carbohydrate is particularly interesting.

We have demonstrated that tumour cell subpopulations of different immunogenicity could be isolated by a method based on their expression of cell-surface carbohydrates[6,7]. In addition, complement-dependent cytotoxicity of serum from tumour-immune animals was inhibited by α-methyl-D-mannoside, a sugar used to elute highly immunogenic cells from con A columns. It seems reasonable that the relative

Table 1 Tumorigenicity of A-10 cells in B6AF$_1$ mice after growth *in vivo* or *in vitro*

Inoculum, growth conditions, host	No. survivors/No. inoculated	Survival time
10^2 Cells, *in vivo* normal host	0/10	23.2±2.3
10^3 Cells, *in vivo* normal host	0/10	21.0±1.8
10^2 Cells, *in vitro* normal host	5/10	27.1±0.8
10^2 Cells, *in vitro* immunosuppressed host	0/10	23.0±0.6
10^2 Cells, *in vitro* to *in vivo*, 1st passage, normal host	4/5	33.0
10^3 Cells, *in vitro* to *in vivo*, 1st passage, normal host	3/5	31.0
10^2 Cells, *in vitro* to *in vivo*, 2nd passage, normal host	0/5	24.2±0.6
10^3 Cells, *in vitro* to *in vivo*, 2nd passage, normal host	0/5	18.6±1.7

Tumorigenicity of A-10 cells was determined by transplantability into B6AF$_1$ mice. Tumour cells grown *in vivo* were washed once in 1% ammonium oxalate (to lyse erythrocytes), followed by two washes in PBS. Cells grown *in vitro* were collected from logarithmic-phase cultures by a rubber policeman, followed by two washes in PBS. Cells for all experiments were >90% viable, as judged by Trypan blue exclusion. Immunosuppression of B6AF$_1$ mice was by whole body irradiation, 450 rad, 24 h before injection of tumour. Intraperitoneal injections contained the appropriate number of cells in 0.2 ml of PBS. Survival time of mice represents the arithmetic mean ± s.e.

amount of mannose-like carbohydrates on the cell surface could influence the antigenic expression of tumour cells.

On the basis of our observations, together with the evidence of heterogeneity in tumour cell populations[7,16], we can speculate on the tumorigenicity of A-10 adenocarcinoma. The subpopulation eluted with PBS comprised a substantial proportion of A-10 tumour grown *in vivo* (45%–50%) and was absent from cultured cells. But the presence of this subpopulation was not necessary for tumour growth *per se*, for cultured cells grew in immunosuppressed mice. This subpopulation may therefore be analogous to the phenotypic expression of epiglycan, and decreased expression of tumour-associated antigens; concomitant with a decreased proportion of mannose-like moieties at the cell surface.

The subpopulations of cells released by sugar, predominantly a feature of cultured cells, would be the inverse analogue of the above situation. The increased proportion of cell-surface con A-binding moieties could result in an increased expression of tumour-associated antigens and decreased tumorigenicity, except in the immunosuppressed host. We observed a 300-fold increase in the agglutinability of A-10 cells by con A after the cell line was established *in vitro*, which was consistent with increased expression of mannose-like moieties (J.J.K., unpublished results). These subpopulations would be selected against by passage of tumour *in vivo*, and the PBS-eluted phenotype would become dominant (Fig. 2). Tumorigenicity would be restored in the normal host.

Although more complex explanations are possible, the concept of tumour-cell subpopulations which depend on tumour-cell environment may have implications for the way in which tumour-cell properties ultimately influence the relationship between tumour and host.

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Characterisation of Aleutian disease antigens

ALEUTIAN DISEASE (AD), which affects certain *Mustelidae*, is a persistent viral infection with development of non-neutralising antibody and gammapathy[1–3]. Some of the antigens developed in the course of AD are detectable by counter-immunoelectrophoresis (CEP) using sera from chronically infected mink. We have obtained evidence of two antigenic forms, Ag-1 and Ag-2, which have been characterised by molecular size, primary protein subunit components and by electron microscopy. Hollow ring-like structures, similar to those described with such viruses as φX174[4], rat virus, Haden, adeno-associated virus and others[5], were found associated with the AD virus (ADV) and seem to be the predominant antigenic component of Ag-1.

The CEP antigens, Ag-1 and Ag-2, were prepared from liver homogenates of ADV-infected mink by repeated Freon extraction and pelleting as described by Cho and Ingram[6]. These extracts were analysed by CEP at each step during the re-extraction procedure. Subsequently, samples rich in Ag-1 and Ag-2 were analysed by velocity sedimentation, molecular filtration and equilibrium density sedimentation. Further analyses consisted of electron microscopy and SDS–polyacrylamide gel electrophoresis of purified antigens.

Electrophoresis of antigens Ag-1 and Ag-2 by CEP revealed that they had different electrophoretic mobilities (Fig. 1). When Ag-1 and Ag-2 were mixed in varying proportions (threefold serial dilutions of Ag-2 and a constant amount of Ag-1), they consistently showed two precipitin bands when resolved by CEP against pooled AD antisera. CEP patterns revealed that Ag-2 was gradually lost during preparative steps consisting of repeated Freon extraction and pelleting; whereas Ag-1 persisted in these conditions. This formed the basis for the partial fractionation of Ag-2 and Ag-1 since Ag-2 was the predominant component in

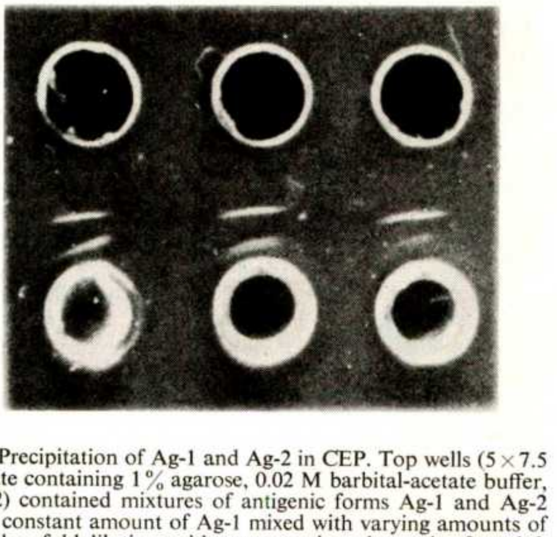

Fig. 1 Precipitation of Ag-1 and Ag-2 in CEP. Top wells (5×7.5 cm plate containing 1% agarose, 0.02 M barbital-acetate buffer, *p*H 8.2) contained mixtures of antigenic forms Ag-1 and Ag-2 with a constant amount of Ag-1 mixed with varying amounts of Ag-2 (threefold dilutions with concentrations decreasing from left to right). The bottom wells contained AD antisera. Ag-1 and Ag-2 were prepared from livers of pastel mink infected 8 d previously with 1 ml 10% w/v liver suspension, Connecticut strain ADV. The method of Cho and Ingram[6] was used which consisted of homogenisation, low speed centrifugation, and three repeated extractions of supernatants with Freon (Du Pont, trichlorotrifluoroethane). The antigens were pelleted from the aqueous phase by high speed centrifugation (180,000*g* for 90 min) to yield Ag-2. Further repeated Freon extraction (1 part Freon, 3 parts antigen solution) followed by high speed centrifugation yielded only Ag-1 activity. The Ag-1 was further purified by equilibrium density centrifugation (CsCl); fractions containing peak Ag-1 activity (1.33–1.35 g cm^{-3}) were dialysed and used in the above precipitations.

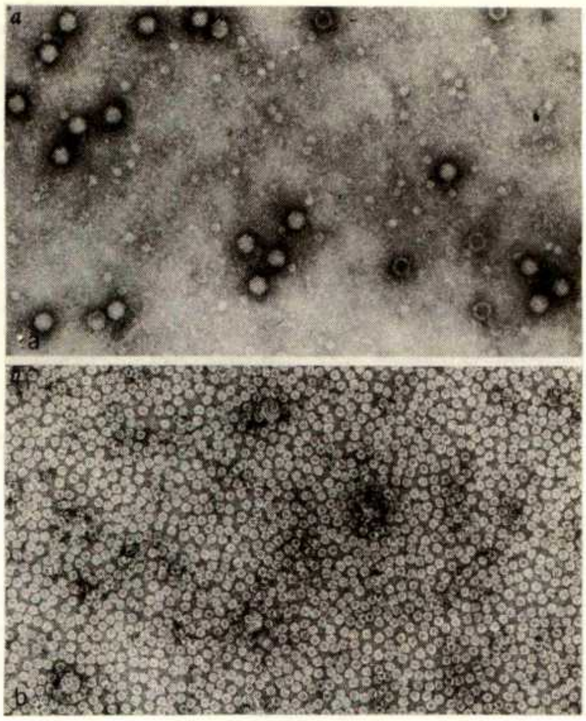

Fig. 2 *a* and *b*, Electron micrographs of Ag-1 component (pooled CsCl fraction 1.33–1.35). *a*, Icosahedral virus particles as well as ring-like structures (stained with sodium phosphotungstic acid, *p*H 7.0). *b*, Similar material stained with uranyl acetate, *p*H 4.2. Magnification: *a*, 100,000; *b*, 117,500.

intermediate (three times) Freon extracted preparations. The relative titre of Ag-2 in these preparations was about 100-fold greater than Ag-1 when estimated by endpoint titrations. On the other hand, after five extractions with Freon, Ag-1 was the only detectable antigenic structure which formed a precipitin band. Although the antigenic forms, Ag-1 and Ag-2, differed in mobility in CEP, they seemed to have similar molecular weights of 2.5×10^6 (gel filtration through Biogel A-15m) and similar sedimentation coefficients (80S).

Ag-1 was further purified by density gradient centrifugation in CsCR as described in Fig. 1. Fractions containing peak antigenic activity (1.33–1.35 g cm^{-3}) were pooled and used for electron microscopy (Fig. 2) and for polyacrylamide gel electrophoretic analyses (Fig. 3).

Electron micrographs of the CsCl fractions containing the CEP antigen showed some intact particles of 27 nm and a large population of ring structures of 13 nm (Fig. 2). Cho and Ingram[7] have also described both ring structures and intact ADV particles. The intact particles cannot be the predominant CEP antigen, Ag-1, because of the low sedimentation coefficient (80S) of Ag-1. Since there were practically no empty particles in the preparation which otherwise could have accounted for this 80S antigen, it seems that the ring structures comprised the CEP antigen, Ag-1. We cannot say whether these structures are generated from intact or defective virus particles as a result of isolation procedures or whether they have accumulated as the result of defective assembly.

The subunit components of Ag-1 as determined by SDS–polyacrylamide gel analysis had molecular weights of 30,000, 25,000 and 15,000 (Fig. 2), which, if they are viral coded, are typical of a picornavirus polypeptide configuration[8] rather than a parvovirus polypeptide pattern, since parvoviruses usually have subunits of molecular weight >50,000 (ref. 9). Since there is always a possibility of cleavage occurring in the primary protein subunits of Ag-1, by the procedures used here for iodination of Ag-1, classification of the Ag-1 as a derivative of a parvovirus or picornavirus would have to be attempted by independent methods. Ag-1 purified from mink infected with the Canadian ADV isolate (F. J. Depauli Ontario Veterinary College, Guelph) showed a polypeptide composition of similar molecular weights. We are pursuing comparative structural and serological characterisation of the subunit proteins of Ag-1 from different strains of ADV. It is conceivable that different strains of ADV may be distinguished on the basis of their subunit compositions.

Ag-2 is a fragile structure which does not produce a discrete band in CsCl density gradients (1.2–1.5 g cm^{-3}) and has not yet been characterised by electron microscopy. The acrylamide gel analyses of Ag-2 (Fig. 2) showed the presence of only one protein subunit of molecular weight 15,000.

Antigenic activity of Ag-2 was not detectable after RNase treatment but was after DNase; whereas Ag-1 was resistant to both. Incubation of Ag-2 with 10 $\mu\text{g ml}^{-1}$ RNase at 37 °C for 30 min completely destroyed the Ag-2 CEP antigenic activity. It has been suggested that the δ peptide of picornavirus is associated with virion RNA. It is conceivable that Ag-2 represents such a viral substructure; however, it could also represent an association of viral protein with host RNA. The only antigenic form that could be isolated from

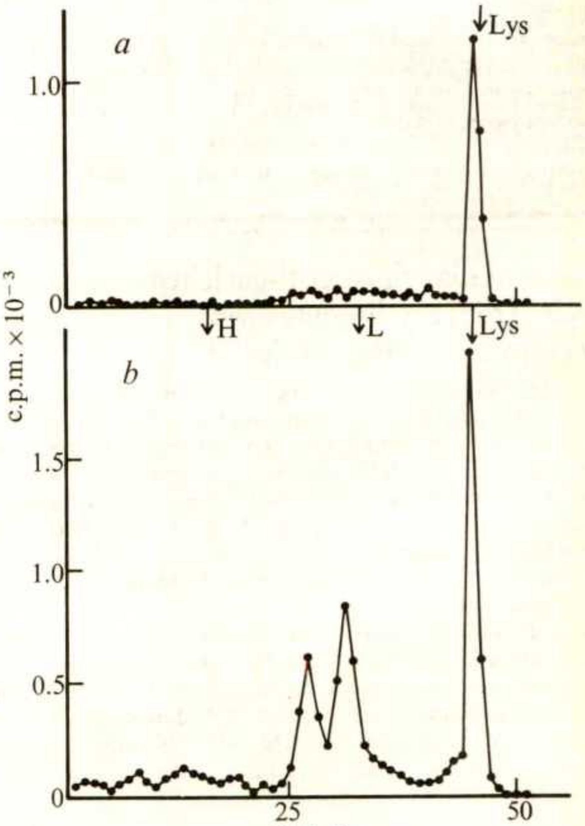

Fig. 3 Electrophoresis of dissociated ^{125}I-labelled Ag-2(*a*) and Ag-1(*b*) on 10% SDS–polyacrylamide gels. Aliquots of Ag-1 and Ag-2 (shown in Fig. 1) were labelled with Na^{125}I using the method of Greenwood *et al.*[10] and dialysed against 0.02 M barbital-acetate buffer, *p*H 8.2, and precipitated in CEP by antibody. The CEP plates were then extensively washed with PBS until free of unreacted components (established by autoradiographs). The precipitin bands were cut out, heated sufficiently in a water bath to melt the agarose and diluted in dissociation buffer (8 M urea–3% SDS at *p*H 8.0) before analysis on SDS–polyacrylamide gel electrophoresis. These precipitin bands were reduced and alkylated[11] in the dissociating buffer and dialysed for 12 h in Tris-HCl buffer, *p*H 6.8, before SDS–polyacrylamide gel electrophoresis[12]. The total gel was cut into 51 slices and counted in a Nuclear Chicago counter. Gels containing samples were compared with standards of human IgG heavy (H) and light (L) chains and lysozyme (Lys) run separately for molecular weight computation.

mink naturally infected in ranch conditions was Ag-2. Further studies on biological and immunological activities of Ag-2 would help in determining whether its accumulation has a role in the pathogenesis of chronic AD.

In these studies we have described properties of two CEP antigens; one is associated with ring structures and the other, which seems to be a nucleoprotein, is present in large amounts especially in mink with chronic AD. Protein subunits of these antigens can be obtained in sufficient purity for primary structural characterisations. Characterisation of these proteins would enable us to determine the relationship of AD virus to other viruses and to establish a basis for the unique biological properties[2] of different ADV strains.

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Release of diacylglycerol-enriched vesicles from erythrocytes with increased intracellular [Ca^{2+}]

WHEN the intracellular Ca^{2+} concentration of human red cells was increased by treatment of the cells with A23187, a divalent cation ionophore, they changed shape from discocyte to echinocyte[1–3] and the 1,2-diacylglycerol content of their membranes was increased[2–4]. In these experiments it seemed that microvesicles budded off from the microvillus-like projections on the echinocytes[2,3]. We have now isolated the microvesicles and examined their composition and structure.

Packed washed human red cells (100 ml), isolated from blood approximately 1 week after collection into acid citrate–dextrose were incubated for 90 min at 37 °C with 10 ml of a medium containing A23187 (2 μg ml^{-1}), 0.154 M NaCl, 2 mM $CaCl_2$ and 10 mM HEPES–NaOH buffer, *p*H 7.4. Cells were sedimented and washed with 90 ml of the same medium. They were then stored at 2 °C for 16 h and were again sedimented and washed. During these procedures cell lysis was about 3%. The two supernatants were centrifuged separately at 20,000*g* for 30 min and each yielded a bright red pellet overlaid by a pink fluffy layer (probably the membranes from lysed cells). The fluffy layer was removed carefully by aspiration and discarded; the red pellets were retained. No additional material was sedimented by centrifugation at $10^5 g$ for 60 min. Cells subjected to the same treatment in the absence of A23187 showed slightly more lysis, but centrifugation of their incubation medium yielded only traces of red pellet. Subsequent analysis showed no significant differences between the red pellets obtained from A23187-treated cells after 90 min and 16 h, and we concluded that these were successive collections of the same type of material and they could be pooled.

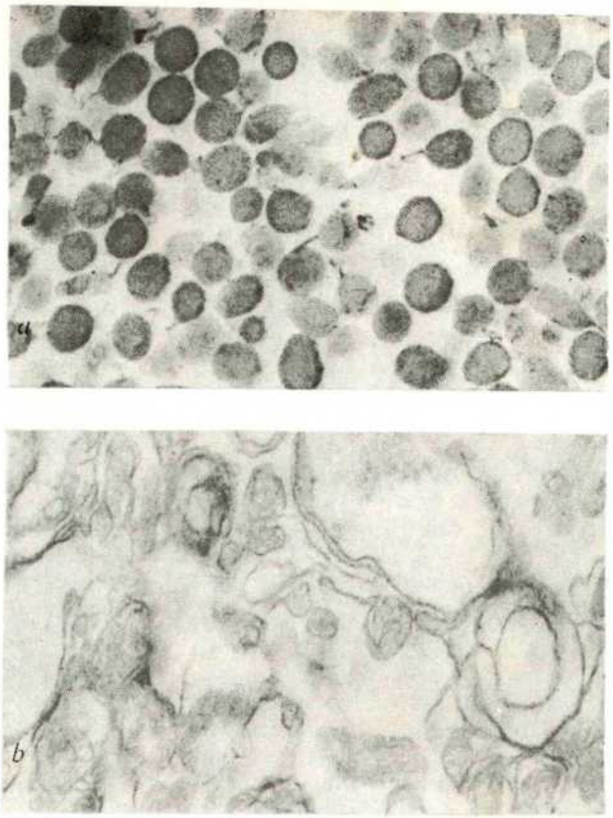

Fig. 1 Electron micrographs of intact microvesicles (*a*), and membranes isolated from glycol-lysed microvesicles from human erythrocytes treated with Ca^{2+} and ionophore A23187 (*b*). Samples were fixed with 4% glutaraldehyde followed by 1% OsO_4. Thin sections of material embedded in Epon were stained with uranyl acetate and lead citrate before examination in a Philips EM301 electron microscope. (×57,000).

Table 1 Lipid analysis of human erythrocytes and microvesicles derived from them

	Untreated cells (4)*	Cells treated with Ca^{2+} and ionophore Residual cells (4)*	Microvesicles (6)*
Phospholipid	56±2†	56±2	54±2
Cholesterol	44±2	44±2	45±2
1,2-Diacylglycerol	0.05±0.025	0.3±0.05	0.8±0.05
% Total phospholipid in subfractions	100	81±5	18±3
Phospholipid class			
Sphingomyelin	25.8±1.7‡	26.7±1.9	28.5±1.7
Phosphatidylcholine	32.0±1.6§	30.7±0.8	27.8±1.4§
Phosphatidylserine	10.5±0.9	10.5±0.1	12.0±1.2
Phosphatidylethanolamine	32.0±1.6	31.6±1.5	32.7±1.2

*Figures in parentheses refer to the number of samples.
†Values are given as mol % of total lipids excluding glycolipids ± s.d.
‡Values are given as mol % of total phospholipids ± s.d.
§The difference between these values is statistically significant ($0.05 > P > 0.02$).
Lipids were extracted and analysed as described previously[4]. Cholesterol was determined by the method of Brown *et al.*[18].

Electron microscopy showed that the pellets consisted mainly of small spherical bodies about 100 nm in diameter with an internal electron density similar to that of erythrocytes (Fig. 1*a*); these are the characteristics expected of microvesicles budded off from the microvilli of A23187-treated cells[3]. When the pellet was frozen and thawed and then subjected to glycol-induced lysis[5], more than 90% of its haemoglobin was released, and the remaining particulate material was sedimented, by centrifugation at 70,000*g* for 30 min, as a pale pink pellet consisting of membrane fragments, some of which seemed to be larger than the profiles of the original vesicles (Fig. 1*b*).

The ratio of total lipid (phospholipid plus cholesterol) to protein for the microvesicle membrane fraction was approximately 1.4 mg lipid per mg protein but, since haemoglobin accounted for about half of the remaining protein, the ratio of lipid to non-haemoglobin protein was about 2.8. The equivalent value for haemoglobin-free erythrocyte ghosts is about 0.7 mg lipid per mg protein[5]; the microvesicle membranes were therefore markedly depleted in protein during their formation and separation from erythrocytes.

Lipid and polypeptide analyses were made on the original cells, on the A23187-treated cells remaining after the liberation of the microvesicles, and on the microvesicle fraction. The latter fraction contained 15–20% of the total cholesterol and phospholipid of the original cells, but less than 1% of their cytoplasm, as defined by haemoglobin content: this is the result expected from the much higher surface to volume ratio of vesicles compared with erythrocytes. As reported previously[2-4], there was a marked increase in 1,2-diacylglycerol content in cells with an increased intracellular Ca^{2+} concentration. The change was even more pronounced in the microvesicles: this fraction contained only a fifth of the cell lipids but almost half of the newly formed diacylglycerol (Table 1). In addition, the microvesicle fraction was depleted in phosphatidylcholine by about 12% compared with the starting material, consistent with the suggestion that phosphatidylcholine is the substrate for Ca^{2+}-stimulated diacylglycerol production[2-4].

As expected from its low protein to lipid ratio, the microvesicle fraction was deficient in polypeptide material compared with the normal membranes (Fig. 2*a* and *b*). This deficiency was due mainly to the almost complete loss of certain components which are prominent in normal membranes, particulaly bands 1 and 2 (spectrin) and band 5 (actin)[6,14]. Periodate–Schiff staining of gels revealed that the major glycoprotein component of the erythrocyte (glycophorin) was also missing from the microvesicles. In contrast, band 3 was prominent both in normal membranes and in the microvesicles (Fig. 2*a* and *b*). Acetylcholinesterase, an enzyme which occupies a superficial site on the surface of normal red cells, was also retained in the microvesicles. Some polypeptides of the microvesicle fraction were cytoplasmic components which had passed into the budding microvesicles; comparison of Fig. 2*b* and Fig. 2*c* suggested that, in addition to haemoglobin, these may have included bands 7 and 8, and possibly band 4.3. In some experiments in which cells were isolated and treated with A23187 within 1 d of collection of the blood, the microvesicles were markedly depleted even of band 3: it is not known why the membrane protein complement of the microvesicles was sensitive to the period of storage of the blood before its exposure to ionophore.

The microvesiculation which occurs in response to increased intracellular Ca^{2+} seems likely to be caused by the increase in diacylglycerol content in the erythrocyte membrane, particularly since the microvesicles themselves have the highest content of this lipid. Outward vesiculation involves membrane fusion through interactions at the cytoplasmic surface of the microvillus-like projections formed initially. Diacylglycerol, which is a fusogenic lipid[7,8], is probably produced in the inner surface of the membrane bilayer where it could mediate the fusion of cytoplasmic surfaces. Neither cell–cell fusion nor internal vesiculation, both of which involve fusion of outer membrane faces, was seen with ionophore treatment. This contrasts with the situation where diacylglycerol is formed in the outer lipid leaflet of erythrocyte membranes using exogenous phospholipase C, when inward vesiculation occurs[9]. These observation emphasise the asymmetrical nature of diacylglycerol-associated membrane budding processes. The asymmetry of the budding reaction probably arises because of the curvature induced by insertion of diacylglycerol into one leaflet of the lipid bilayer; the contraction in area of this leaflet may be a direct consequence of the formation of diacylglycerol[10]. The asymmetry is probably preserved only for as long as a gradient of the fusogenic molecule is maintained between the two lipid leaflets of the membrane. For diacyl-

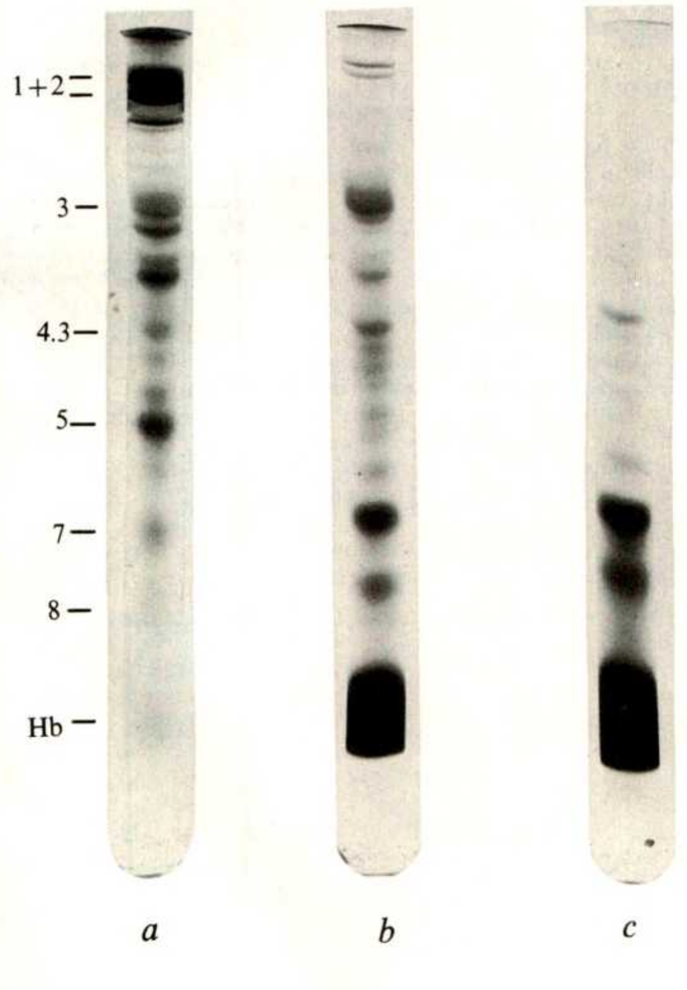

Fig. 2 Sodium dodecyl sulphate (SDS)–polyacrylamide gels of: *a*, human erythrocyte ghosts prepared by glycol lysis[5]; *b*, microvesicles released from human erythrocytes treated with Ca^{2+} and ionophore A23187; *c*, cytosol fraction prepared by centrifugation of osmotically lysed erythrocytes at 15,000*g* for 15 min. *a* and *b* contained 35 nmol of phospholipid and *c* contained about 300 µg of haemoglobin. Samples were dissolved in 10% sucrose, 4% SDS, 1% β-mercaptoethanol, 10 mM phosphate buffer, *p*H 7.0, and heated at 95 °C for 1 min before application to 6% polyacrylamide gels. Electrophoresis was carried out at 4 mA per gel, using a buffer consisting of 100 mM sodium phosphate, *p*H 7.0, containing 0.1% SDS. Gels were stained with Coomassie blue.

glycerol it seems likely that equilibration between the two leaflets is fairly rapid[9]. Heating or prolonged incubation would assist equilibration between the two leaflets and this may explain why A23187-treated cells, which do not fuse immediately at 37 °C, can be made to fuse at 47 °C or on prolonged storage[11].

The microvesicle membranes were markedly deficient in protein relative to the original erythrocyte membrane, suggesting that membrane fusion leading to outward vesiculation is more likely to occur in protein-depleted areas. This finding is consistent with a general model of membrane fusion proposed by Lucy *et al.*[12]. This model, however, envisaged the removal of all intrinsic proteins from the area of the membrane involved in fusion, whereas our data demonstrate that at least one major intrinsic protein (band 3)[13] is still present in the microvesicle membrane. Furthermore, the proteins most notably absent from microvesicles are spectrin and actin, extrinsic proteins which form a meshwork covering the inner surface of the membrane[14] where they might prevent fusion of lipid regions. It may be necessary for such extrinsic proteins to be removed from membranes before they become able to fuse.

Perhaps by virtue of their modified composition, particularly their decreased protein and increased diacylglycerol content, the microvesicle membranes may be prone to fuse with one another; this would contribute to the formation of extended membrane systems from microvesicles subjected to freeze–thawing, lysis and ultracentrifugation (Fig. 2*b*).

It has been suggested[12,15] that production of fusogenic lipids *in situ* might play a part in physiological membrane fusion events such as exocytosis or virus-induced cell fusion. The physiological role of diacylglycerol production at the inner surface of the erythrocyte membrane by an endogenous Ca^{2+}-stimulated phospholipase C is not understood, but is clearly associated with echinocytosis and outward vesiculation. Such vesiculation, possibly as a result of declining or defective Ca^{2+} homoeostasis, could explain the loss of membrane components which occurs gradually in ageing cells[16] and more rapidly in hereditary spherocytic erythrocytes[17].

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Calcium ions regulate cyclic AMP and beating in cultured heart cells

ALTHOUGH the transport of Ca^{2+} is known to be involved in the control of beating by cultured heart cells, little is known about the regulation of this process. There is evidence that catecholamines affect heart cells through their influence on cyclic AMP[1-4]. In this way they might affect Ca^{2+} transport and, as a result, contractility. Our experiments with beating heart cells now suggest that both noradrenaline and Ca^{2+} control the concentration of cyclic AMP in the cells which, in turn, regulates the utilisation of Ca^{2+}. Addition of noradrenaline or depletion of Ca^{2+} in the external medium results in cells which beat in a lower concentration of external Ca^{2+} compared with controls.

Our experiments were carried out with rat heart cells prepared and cultured as before[5-6]. In a preliminary experiment noradrenaline (10^{-5} M) increased the cellular concentration of cyclic AMP (as determined by the method of Gilman[7]) from 3 to 8 pmol per mg protein within 2 min. It seemed therefore that since cyclic AMP has been reported to increase Ca^{2+} uptake, noradrenaline should also increase the utilisation of Ca^{2+} by beating heart cells. To test this, the beating of heart cells was measured in the presence of different concentrations of Ca^{2+}, decreasing from 8×10^{-3} M, in the presence and absence of noradrenaline (Fig. 1). The rate of beating decreased with the decreasing concentration of Ca^{2+}, and beating stopped at 10^{-4} M Ca^{2+}. In the presence of noradrenaline, however, beating was sustained down to 3×10^{-5} M Ca^{2+}. Thus, noradrenaline increased the ability of the heart cells to utilise Ca^{2+} for beating.

We also studied the effect of Ca^{2+} on the sensitivity of the cells to Ca^{2+} and on the level of cyclic AMP. We measured the concentration of external Ca^{2+} needed to reinitiate beating in heart cells partially depleted of Ca^{2+} by incubation in low Ca^{2+} medium for 2 h. (During this time, membrane-bound Ca^{2+} and most of the easily exchangeable Ca^{2+} is lost[8,9].) Cells ceased beating at a concentration of Ca^{2+} below 2.5×10^{-4} M. In cultures preincubated in 3×10^{-6} M Ca^{2+} medium for 2 h, beating was restored by 4.5×10^{-5} M Ca^{2+}. This is six times less than the concentration of Ca^{2+} needed to maintain the beating of cells preincubated in 'normal' medium, which contains 10^{-3} M Ca^{2+}. In another experiment cells incubated in 4.5×10^{-5} M Ca^{2+} stopped beating as expected, but within 2 h beating resumed spontaneously (Fig. 2). Thus the cells in low Ca^{2+} developed a sensitivity to Ca^{2+} and gained an ability to beat at the lower level. The requirement for external Ca^{2+} was

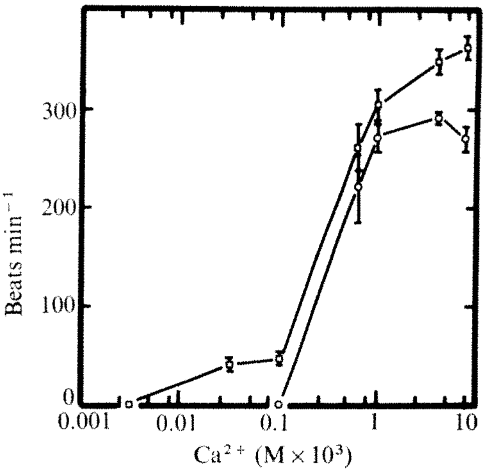


Fig. 1 Effect of noradrenaline (10^{-5} M) and Ca^{2+} on beating of heart cells. Rat heart cells were prepared as before[5,6] and incubated for 2 d in CMRL (1415AM) culture medium[14] supplemented with 5% foetal calf and 5% horse serum. Fresh medium was added 2 h before the experiment. Experimental medium was made with serum previously dialysed with Ca-free Tyrode solution; $CaCl_2$ was added to yield the indicated final Ca^{2+} concentrations, taking into account the basal level of Ca^{2+} measured by atomic absorption spectrophotometry. The rate of beating was determined visually after 20 min of incubation. The temperature was maintained at 37 °C. Data points represent the average of three or four plates; the vertical bars represent standard error of the mean. □, plus noradrenaline; ○, minus noradrenaline.

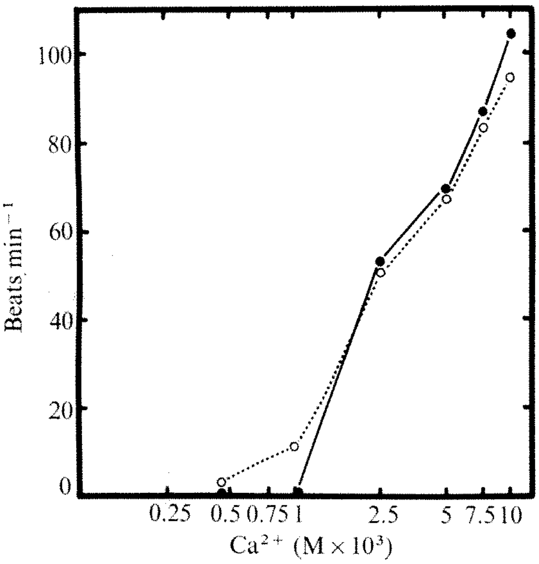
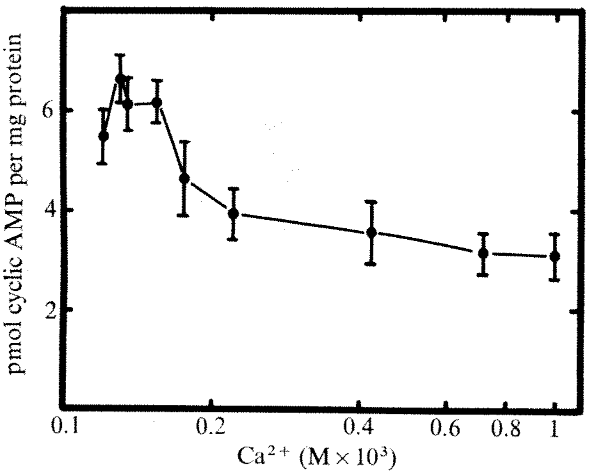


Fig. 2 Beating of heart cells conditioned in high or low Ca^{2+} medium. Two-day-old cultures were incubated in fresh medium for 2 h before the addition of experimental medium (●). Beating rates in duplicate were measured 10 min after treatment. ○, Beating rates of heart cells treated as above but with an additional incubation in 4.5×10^{-5} M Ca^{2+} for 2 h before the addition of experimental medium. The solid symbol at 4.5×10^{-5} M Ca^{2+} indicates the beating rate 10 min after the cells were placed on this medium. The open symbol at 4.5×10^{-5} M Ca^{2+} indicates the beating rate 2 h after the cells were placed on this medium.

quantitatively dependent on previous exposure of the cells to Ca^{2+}. Cells incubated in a medium with high Ca^{2+} had a greater minimum external Ca^{2+} requirement to maintain beating than did cells incubated in a low Ca^{2+} medium. This indicated that the external Ca^{2+} concentration is involved in controlling the effect of Ca^{2+} on beating, and perhaps in controlling the intracellular level of Ca^{2+}.

If cyclic AMP influences Ca^{2+} transport, and in turn the external Ca^{2+} concentration also influences Ca^{2+} transport, it is possible that Ca^{2+} influences the concentration of cyclic AMP. To test this, we measured the direct effect of Ca^{2+} on cyclic AMP. In a preliminary experiment 10^{-5} M noradrenaline increased the concentration of cyclic AMP in cells incubated in 10^{-3} M Ca^{2+} from 3 to 8 pmol per mg protein. Cells in low Ca^{2+} medium responded by an increase of cyclic AMP from 3 to 12 pmol per mg protein. After 1 h the cyclic AMP in cells incubated with noradrenaline in 10^{-3} M Ca^{2+} had decreased to 5 pmol per mg protein, and with noradrenaline in low Ca^{2+} medium it had fallen only to 9 pmol per mg protein. Thus, in cells incubated in a medium containing noradrenaline and a low Ca^{2+} concentration, cyclic AMP reached a greater concentration for a longer time than in cells in medium containing more Ca^{2+}. Similar effects of noradrenaline on cyclic AMP have been reported for renal tubules in the presence of different concentrations of Ca^{2+} (ref. 10).

Fig. 3 Effect of external Ca^{2+} on cellular concentration of cyclic AMP. Two-day-old cultures were incubated for 2 h in growth medium with different Ca^{2+} concentrations, and cyclic AMP was determined. Each point represents an average of four plates. The vertical bars represent standard error of the mean. A t test was performed on grouped cyclic AMP values between 10^{-4} M and 2×10^{-4} M and between 2×10^{-4} M and 10^{-3} M. The level of significance was <0.001.



If cyclic AMP is related to the sensitivity of heart cells to Ca^{2+} then its concentration should respond directly to changes in external Ca^{2+}. When cells were incubated for 2 h in various concentrations of Ca^{2+}, their content of cyclic AMP increased from 3 to 6 pmol per mg protein as the concentration of Ca^{2+} decreased from 10^{-3} M to 10^{-4} M (Fig. 3). Thus a decrease in cellular Ca^{2+} increased the cyclic AMP and decreased the external Ca^{2+} requirement for beating, perhaps through the concomitant increase in cyclic AMP. In this way Ca^{2+} may regulate its own effect on contractility.

Ca^{2+} may affect the concentration of cyclic AMP by an effect on cyclic AMP phosphodiesterase. This enzyme is stimulated by a modulator protein which is activated by Ca^{2+} (ref. 11). It has been suggested that Ca^{2+} is the limiting factor in the normal physiological state. Thus, a decrease in Ca^{2+} would cause a decrease in cyclic AMP phosphodiesterase activity and a resultant increase in cyclic AMP. Brooker[12] has reported that the level of cyclic AMP varies cyclically in relation to the beating cycle. This could be caused by the oscillation in the cytosol Ca^{2+} level which results from increased uptake of Ca^{2+} by the sarcotubular vesicles during the diastolic part of the beating cycle. It is also interesting that we have evidence that the Ca^{2+} protein activator of cyclic AMP phosphodiesterase is located on the sarcoplasmic reticulum[13]. Ca^{2+} has multiple roles in cardiac contractility, and we suggest that it has a role in regulating its own utilisation, perhaps through its control of cyclic AMP.

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Alteration of electrical activity in molluscan neurones by cyclic nucleotides and peptide factors

ELECTRICAL activity in certain molluscan neurones is subject to long lasting synaptic and hormonal modulation[1-5]. Evidence from several systems suggests that cyclic nucleotides mediate alterations in membrane properties in nerve and muscle cells[6-8]. We report here that, in certain neurones from the marine snail *Aplysia californica* and the land snail *Helix pomatia*, an increase in the intracellular concentration of cyclic nucleotides is accompanied by long lasting changes in electrical activity, and that physiological agents known to modify the electrical rhythms of these nerve cells, increase adenosine 3′,5′-cyclic monophosphate (cyclic AMP) in *Helix* and *Aplysia* nervous tissue.

Table 1 Effect of phosphodiesterase inhibitors on cyclic AMP in *Aplysia* abdominal ganglion

Treatment	Cyclic AMP (pmol per mg protein)
Control	14±2 (9)
Papaverine, 10^{-5} M	22±2* (4)
Papaverine, 3×10^{-4} M	29±4† (5)
IBMX, 10^{-5} M	38±6† (4)

Aplysia abdominal ganglia were preincubated for 30 min at 20±1 °C in control medium, then incubated for an additional 30 min in either control medium or medium containing the indicated concentration of inhibitor, before assay of cyclic AMP. After incubation, abdominal ganglia were frozen in liquid N_2, and homogenised immediately in acidified ethanol[21]. Homogenates were centrifuged to precipitate protein, and the ethanol supernatants were dried under reduced pressure. Cyclic AMP was measured by a binding assay[22]. Values are means ± s.e.m. for the number of ganglia shown in parentheses.

*Significantly different from control, $P < 0.05$.

†Significantly different from control, $P < 0.01$.

Identified neurones R15 (ref. 9) in *Aplysia* abdominal ganglion and F–1 (ref. 5) in *Helix* circumoesophageal ganglia exhibit similar patterns of endogenous electrical activity, in which an oscillating membrane potential underlies alternating periods of action potential bursts and interburst hyperpolarisations[10]. We have found that the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX), added to the bath at concentrations between 10^{-5} M and 3×10^{-4} M, has profound effects on the bursting rhythm of these cells (Fig. 1*a*). After a lag of 20–45 min there was a marked increase in both the duration and amplitude of the interburst hyperpolarisation, as well as in the frequency and number of action potentials per burst. Simply shifting basal membrane potential (by intracellular injection of hyperpolarising or depolarising current) did not produce this effect. Similar concentrations of the alkaloid papaverine, another potent phosphodiesterase inhibitor, altered bursting rhythm in the same way as IBMX. These results suggested that an increase in concentrations of cyclic nucleotide might be involved in the change in electrical pattern; we found (Table 1) that cyclic AMP did accumulate in the *Aplysia* abdominal ganglion after treatment with IBMX or papaverine.

The action of IBMX and papaverine on bursting rhythm in neurones R15 and F–1 was not mimicked by externally perfused cyclic AMP or cyclic GMP in concentrations as high as 2.5×10^{-3} M. This might have been due to their failure to penetrate the cell membrane, or to their rapid destruction by intracellular phosphodiesterases before they reach the site of action. To circumvent the problem of membrane permeability, we injected solutions of cyclic AMP or cyclic GMP (2.5×10^{-3} M in water, adjusted to *p*H 7.4) into R15 and F–1 through intracellular micropipettes[11]. Approximately 1 nl of solution was injected, and intracellular dilutions of 10–20-fold can be assumed. No change in bursting rhythm was observed in three preparations injected with cyclic AMP and two injected with cyclic GMP, indicating that low permeability is not sufficient to explain

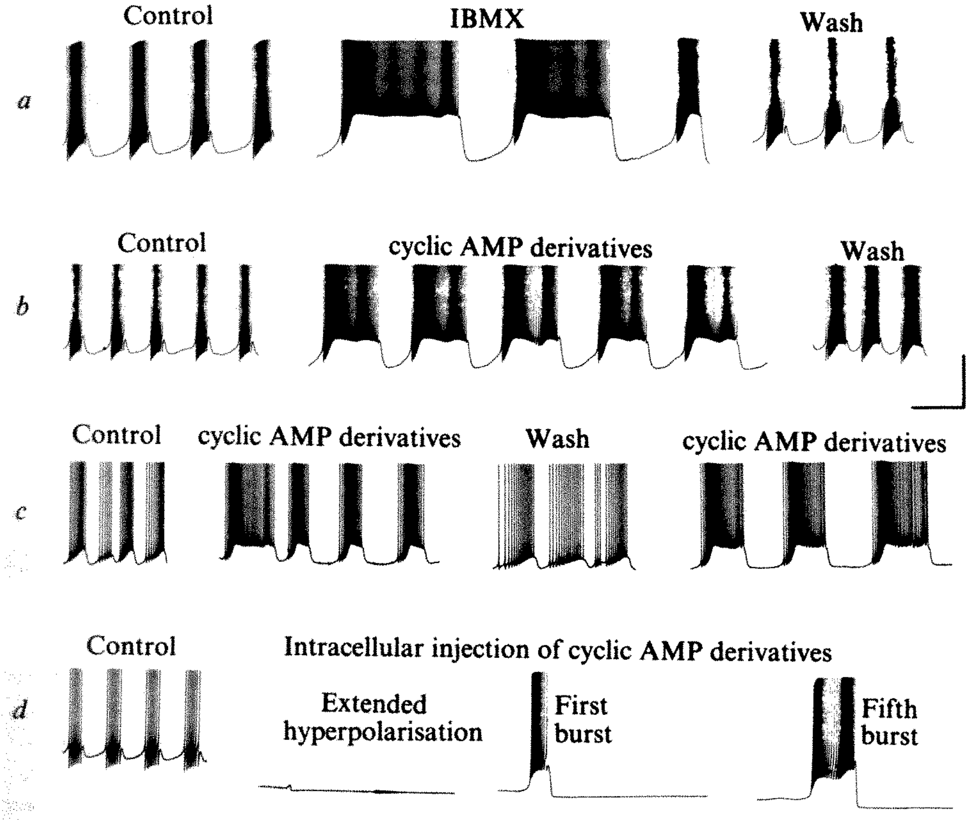

Fig. 1 Effects of IBMX and cyclic AMP derivatives on electrical activity in neurones R15 and F–1. Intracellular recording micropipettes were filled with either 3 M KCl or 0.6 M K_2SO_4. All experiments were performed at 20±1 °C, between 0800 and 2100. *a*, Electrical activity was measured in cell R15 in an *Aplysia* abdominal ganglion during perfusion with normal *Aplysia* medium[21] (control), then 45 min after addition of *Aplysia* medium containing 3×10^{-4} M IBMX, and finally normal medium again (wash). *b*, Same as (*a*) except that a mixture of 8-benzylthio cyclic AMP and 8-(4-aminobutylamino) cyclic AMP (10^{-3} M each, obtained from ICN, Cleveland) was used in place of IBMX. *c*, Electrical activity measured in cell F–1 in *Helix* nervous system perfused with normal *Helix* medium (control), then *Helix* medium containing a mixture of cyclic AMP derivatives as in (*b*). After a 90-min wash with normal medium the derivatives were reintroduced. *d*, Effects of injection into R15 of the mixture of cyclic AMP derivatives (10^{-3} M each dissolved in water adjusted to *p*H 7.4). Within 6 min the cell hyperpolarised to a level 23 mV below that of the pre-injection interburst hyperpolarisation level, and remained silent for 30 min. When bursting returned, the pattern was similar to that observed after application of phosphodiesterase inhibitors and cyclic AMP derivatives to the bath. Shown are the first and fifth (18 min after first) bursts after the extended hyperpolarisation. Calibration: 40 mV, 20 s.

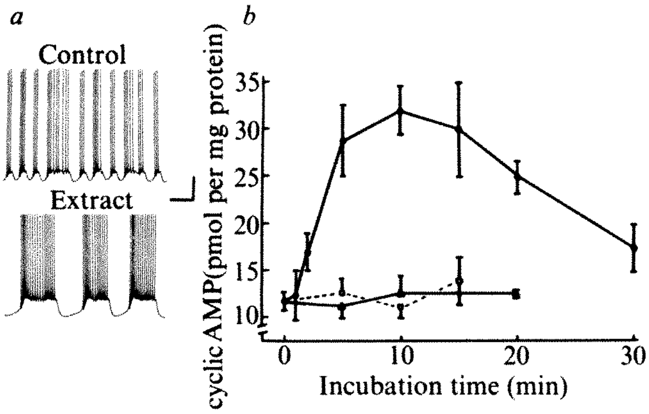

Fig. 2 Effects of *Helix* circumoesophageal ganglia extract on neuronal membrane properties and concentrations of cyclic AMP in *Helix* nervous system. Ganglia were removed from active[25] *Helix*, placed in 5-ml beakers containing 1 ml of *Helix* medium (modified from that described previously[2] by addition of amino acids and vitamins[21]), and preincubated at 20±1 °C for 30 min before the start of the experiment. Acetic acid extract from 2.5 *Helix* circumoesophageal ganglia (total tissue wet weight 59 mg, total tissue protein content 1.6 mg), prepared as before[2] and lyophilised, was resuspended in 1 ml of *Helix* medium as test substance. Ganglia were incubated in medium either with or without extract, were frozen in liquid N_2 and homogenised immediately in acidified ethanol. Homogenates were treated as for Table 1. Concentrations of cyclic AMP were measured by a binding assay[21]. The same resuspended extract was used for both (*a*) and (*b*). *a*, Membrane potential traces of cell F–1 before (top) or 20 min after (bottom) replacement of control bathing medium with extract-containing medium. The change in bursting rhythm induced by the extract could be reversed by prolonged washing in extract-free medium. Calibration: 20 mV, 10 s. *b*, Concentrations of cyclic AMP in circumoesophageal ganglia incubated in control medium (▲) or medium containing extract (●) for various times. Other circumoesophageal ganglia were incubated in 1 ml of medium, containing acetic acid extract of *Helix* foot muscle (total tissue wet weight 263 mg, total tissue protein content 9.4 mg), for various times (□). Data are mean ± s.e.m. for 5–12 samples.

their lack of effect during application to the bath. Accordingly, we examined the effects of the 8-benzylthio, 8-parachlorophenylthio, and 8-(4-aminobutylamino) derivatives of cyclic AMP, all of which inhibit, and are highly resistant to breakdown by, phosphodiesterases[12]. These compounds are also potent activators of mammalian[12] protein kinases, and are more effective than cyclic AMP in altering electricial activity in cerebellar Purkinje cells[13]. All the derivatives, perfused at concentrations ranging from 10^{-5} M to 10^{-3} M, modified the bursting rhythm in cells R15 and F–1 after a 20–45-min lag (Fig. 1*b* and *c*). The effect was observed in all ten cells tested, and was transitory (although lasting at least 20 min) in six of them, even though the drug remained in the bathing medium. Reintroduction of the appropriate derivative, after a 1-h wash, caused a long-lasting (20 min or longer) reappearance of the modified bursting rhythm, again after a 20–45-min lag. Application of 10^{-3} M $N^6,O^{2'}$-dibutyryl cyclic AMP to the bath had no effect, in agreement with a previous report[14].

The 8-parachlorophenylthio derivative of cyclic GMP, perfused at 10^{-3} M, did not affect the interburst hyperpolarisation in any of four R15 cells tested. Occasionally we noted an increased burst amplitude during treatment with this compound; this occurred preferentially after the long lasting, spontaneous i.p.s.p.s., already documented in R15 (ref. 15). In all four cells, treatment with 8-parachlorophenylthio cyclic GMP was followed by perfusion with the same concentration of 8-parachlorophenylthio cyclic AMP, either with or without an intervening wash. In all cases the cyclic AMP derivative potentiated the interburst hyperpolarisation, previously unaffected by the cyclic GMP derivative.

It is unlikely that the altered bursting rhythm reflects changes in neurones presynaptic to R15 and F–1. All three cyclic AMP derivatives which were effective when applied to the bath were also effective when injected into the cell bodies of R15 and F–1 (Fig. 1*d*). Again, a time lag was observed, suggesting that the lag after external application did not reflect a slow intracellular buildup of the derivatives, but rather was due to some slowly occurring metabolic process, or to diffusion of the derivatives to a site where they can act. Presumably, the membrane of the cell body, the site of action potential generation in bursting molluscan neurones[16], is available to the compounds soon after their injection. In addition, tetrodotoxin-treated *Aplysia* ganglia, in which action potentials are suppressed, but slow oscillatory waves in R15 continue[17], showed a lengthening of both hyperpolarising and depolarising phases of the cycle, when 3×10^{-4} M IBMX was added (our unpublished results). This indicates that the IBMX-induced change in R15 firing pattern (Fig. 1*a*) did not result from an alteration in firing pattern of some presynaptic neurone; it also demonstrates that the modified electrical rhythm did not require action potentials in R15.

Peptides from various sources can markedly alter membrane properties of certain molluscan neurones in a manner similar to that which we observed after exposure to cyclic nucleotides. Active factors include the vertebrate neurohypophysial hormones vasopressin and oxytocin[1,18], as well as peptide-containing extracts from the nervous system of the terrestrial snail *Otala lactea*[2] and *A. californica*[2,3]. This suggests that cyclic AMP is an intermediary in the action of these agents, and we observed the accumulation of cyclic AMP in molluscan nervous system exposed to peptide hormones and peptide-containing extracts, at concentrations similar to those which alter neuronal membrane properties.

The effects of a *Helix* circumoesophageal ganglia extract on neuronal membrane properties and on cyclic nucleotide levels in *Helix* nervous system are shown in Fig. 2. As previously reported for cell 11 of *Otala*[2], the extract markedly increased the depth and duration of the interburst hyperpolarisation, as well as the number and frequency of action potentials per burst, in cell F–1 of *Helix* (Fig. 2*a*), a result similar to that observed after addition of cyclic nucleotides to the medium bathing the nervous system. The same concentration of extract caused a large increase in cyclic AMP in *Helix* circumoesophageal ganglia (Fig. 2*b*). Concentrations of cyclic AMP were increased after as little as 2 min of incubation, reached a maximum at about 10 min, and

Table 2 Effect of *Aplysia* bag-cell extract on cyclic AMP in *Aplysia* nervous system

Tissue fraction	Cyclic AMP (pmol per mg protein) Control	Bag-cell extract
Neurone R2 soma	23±1 (9)	20±3 (8)
Neurone R15 soma	16±6 (9)	19±6 (9)
Left upper quadrant of AG	14±3 (8)	38±8* (9)
Remainder of AG	12±1 (8)	30±5* (8)
Connective nerves	15±3 (5)	13±4 (5)

Acetic acid extracts of clusters of neurosecretory neurones (bag cells) from abdominal ganglia (AG) of *Aplysia* were prepared as described by Ifshin *et al.*[2]. The extracts were lyophilised and resuspended in appropriate volumes of *Aplysia* medium. AG were preincubated in *Aplysia* medium for 30 min at 20±1 °C, then incubated in control medium or 1 ml of medium containing extract from four bag-cell clusters per ml, for 10 min before dissection. AG were then frozen in ethylene glycol-dry ice[23], and identified neuronal cell bodies and segments of the AG were dissected and homogenised in acidified ethanol. Homogenates were treated as described for Table 1. R15 somata were assayed by radioimmunoassay[24], other fractions by binding assay[21]. Values are means ± s.e.m. for the number of samples shown in parentheses.

*Significantly different from control, $P < 0.01$.

returned to near basal levels by 30 min. The factor in the extract responsible for the increase in cyclic AMP was stable at 100 °C or in 50% acetic acid, eluted in the exclusion volume of a Sephadex G-10 column and was destroyed by Pronase. In all these respects it is identical to the factor, tentatively identified as a peptide, which alters bursting rhythm in *Otala* cell 11 (ref. 2). An acetic acid extract of snail foot muscle affected neither bursting rhythm[2] nor concentrations of cyclic AMP (Fig. 2*b*).

The demonstration that an *Aplysia* bag-cell extract alters bursting rhythm in cell R15 of the abdominal ganglion[3], and that cyclic nucleotides can mimic this effect, led us to investigate the influence of such an extract on cyclic AMP in different regions of the abdominal ganglion. No change in concentrations of cyclic AMP was observed in the cell bodies of the silent neurone R2 or the bursting neurone R15 after incubation of the abdominal ganglion with extract (Table 2). There was, however, a large increase in cyclic AMP in the left upper quadrant of the abdominal ganglion (which is rich in bursting neurones[19]), as well as in that portion of the ganglion remaining after removal of the R2 and R15 cell bodies and the left upper quadrant. The lack of any accumulation of cyclic AMP in the somata of R2 and R15 suggests that the response is confined to the neuropil, which contains both neuronal processes and glia[20]. Since connective nerves which are rich in stellate glial cells[20] did not accumulate cyclic AMP in the presence of bag-cell extract (Table 2), it is unlikely that this class of glia is involved.

Our findings are all consistent with the possibility that cyclic AMP mediates the effects of peptides on bursting rhythm, and may play a role in modulating the membrane properties of certain nerve cells.

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N-demethylation of morphine in rat brain is localised in sites with high opiate receptor content

N-DEMETHYLATION of morphine, a principal biotransformation of this opiate alkaloid, has been demonstrated in several species including man[1–4]. The primary site of the reaction is the liver[5,6] but its existence in the central nervous system (CNS) has been inferred from experiments both *in vitro* with rat brain slices[7], and *in vivo* with hepatectomised rats[8]. The enzymatic *N*-demethylation of morphine and other biologically active tertiary amines has been studied extensively and both Axelrod[5,6] and Beckett *et al.*[9] have proposed that *N*-demethylation of narcotics is intimately involved in the mechanism of analgesia and the development of tolerance to and dependence on these agents. Subsequently, other investigators have questioned the validity of these hypotheses[10,11] and critical reviews of the experimental support of these theories have been published[12,13]. Belleau and Morgan[14] have revived interest in the biological significance of the *N*-demethylation of narcotics by proposing a provocative concept of clastic binding to opiate receptors in which the process of *N*-demethylation participates in conjunction with the opiate receptor in the mechanism of opiate action. This concept requires that the location of the sites of *N*-demethylation within the CNS is proximal to or coincident with that of the opiate receptors. Previous reports[7,8,15] have described the *N*-demethylation of morphine by the whole brain, and our study was initiated to locate the reaction at specific sites. To do this *in vivo,* we used a new, highly sensitive assay. A mixture of 6-^{3}H-morphine[16], and *N*–^{14}CH$_3$-morphine (Amersham/Searle), of known isotope ratio, was injected into each rat and, after killing, the isotope ratio of selected tissues was determined accurately by combustion in a tissue oxidiser. *N*-demethylation of this mixture generates ^{14}C-containing fragments originating from the *N*-methyl group, but the *N*-demethylated products of the reaction contain only ^{3}H. The former are dissipated from the site of reaction much more rapidly than the latter, so that an increase in the ratio of ^{3}H to ^{14}C in a specific tissue would serve to indicate the presence, and also the extent, of any *in situ* *N*-demethylation.

The results of a series of experiments are listed in Table 1. The liver is a known site of *N*-demethylation[5,6] and in every study this tissue contains a ratio of ^{3}H to ^{14}C significantly greater than that of the blood and can therefore be considered as a positive control. The only other tissues showing a similar increase in the isotopic ratio were in the CNS and consisted of the hypothalamus, the medial thalamus and the corpus striatum. The ratio of ^{3}H to ^{14}C in these tissues was significantly greater than that in the blood, indicating that the peripherally administered morphine was *N*-demethylated at these sites. In contrast, none of the other tissues examined had a ratio of ^{3}H to ^{14}C greater than that of the blood, suggesting that they were not sites of *N*-demethylation. This evidence, however, need not be exclusive since further dissection of the other brain areas could provide fraction(s) with disproportionate isotope ratios which were obscured by the isotope contents of the larger section in our work.

These results were obtained in experiment 1 in which a pharmacological dose of morphine ($\sim 10\ \mathrm{mg\ kg^{-1}}$) was injected. In experiments 2 and 3, in which tracer or subpharmacological doses of morphine were injected, there were no comparable changes in isotope ratio, in spite of continued clear and undiminished evidence of the *N*-demethylation in the liver. This failure to observe isotope ratio differences in the central tissues in these experiments cannot be due to insensitivity of detection for although the tissues contained less material, the amount of radioactivity present in the CNS regions was comparable with that in experiment 1. This suggests that *N*-demethylation in the brain proceeds only when pharmacological concentrations of the substrate are present, which tends to confirm the biological significance of the reaction. Alternatively, it is possible that morphine is demethylated in the CNS when low doses are present, but that the smaller amounts of *N*-demethylated products formed are lost from the site of the reaction within 30 min, and the loss of both isotopes results in failure to observe changes in ratio. In experiment 4, in which a pharmacological dose of morphine was used but the animals were killed after 60 min, there is significantly

Table 1 Rat tissue isotope ratios after subcutaneous administration of 6-^{3}H-morphine/*N*–^{14}C-morphine (normalised to dose ratio = 1)

Experiment	1	2	3	4	5
Blood	0.89±0.02	0.92±0.05	1.06±0.04	0.92±0.02	1.02±0.07
Cerebral cortex	0.82±0.08	0.73±0.08	0.84±0.09	0.89±0.04	0.85±0.11
Hypothalamus	1.04±0.07	0.83±0.12	0.96±0.10	1.00±0.07	1.22±0.13
	$P < 0.001$			$P < 0.02$	$P < 0.01$
Medial thalamus	1.24±0.25	0.79±0.19	0.92±0.13	0.98±0.07	1.40±0.23
	$P < 0.001$			NS	$P < 0.01$
Corpus striatum	1.15±0.08	0.84±0.10	0.95±0.16	0.99±0.06	1.20±0.07
	$P < 0.001$			$P < 0.02$	$P < 0.001$
Midbrain	0.83±0.04	0.69±0.07	0.87±0.04	0.91±0.07	0.86±0.10
Cerebellum	0.82±0.06	0.77±0.03	0.92±0.12	0.88±0.04	0.93±0.08
Medulla	0.88±0.04	0.82±0.08	0.95±0.10	0.97±0.07	0.97±0.11
Liver	1.20±0.07	1.23±0.04	1.45±0.16	1.25±0.09	1.21±0.05
	$P < 0.001$	$P < 0.001$		$P < 0.001$	$P < 0.001$

The recorded isotope ratios are the average for six male Sprague–Dawley rats (~ 250 g) used in each study. Significance (to blood) was calculated by Student's *t* test[24]. Animals were killed by cervical fracture, and the CNS was dissected as before[25]. Tissues were combusted in an Oxymat tissue oxidiser (Intertechnique). The tritiated water and $^{14}CO_2$ released by each sample was trapped and counted in a Packard scintillation counter. In experiments 1, 2, 3 and 5, animals were killed 30 min after injection; in experiment 4, after 60 min. Doses were: experiment 1, 2.5 mg morphine per rat; 2, 0.2 mg morphine per rat; 3, 0.02 mg morphine per rat; 4, 2.5 mg morphine per rat; 5, 2.5 mg morphine + 0.1 mg naloxone per rat.

less evidence for *N*-demethylation in the hypothalamus, corpus striatum and medial thalamus, presumably due to the greater loss of the tritium-containing *N*-demethylated products from the site of the reaction during the longer time. The isotope ratios in the liver were not affected by the increase in time, suggesting either a continuing reaction or a greater retention time of the *N*-demethylated products in this organ.

The observed isotope ratio changes in specific central tissues could be interpreted to arise not from *in situ* *N*-demethylation of the 6-^{3}H-morphine–N-$^{14}CH_3$-morphine mixture, but as a product of preferential uptake of *N*-demethylated compounds originating from the liver. Several lines of evidence argue against this interpretation. Demethylated (nor-)morphine and presumably other *N*-demethylated products cross the blood–brain barrier less effectively than the corresponding *N*-methyl derivatives[17,18] and this would lead to isotope ratio changes, which would be the reverse of those observed. Furthermore, in unpublished studies in which liver *N*-demethylation was abolished, changes were observed in the isotope ratio in the CNS. Finally, in experiment 4, in spite of continuing liver *N*-demethylation, the changes in isotope ratio in the CNS were reduced substantially with time.

Our work demonstrates that the *N*-demethylation of morphine in the brain of the rat is limited to specific sites which include the hypothalamus, medial thalamus and corpus striatum. These tissues have been reported to contain relatively high concentrations of opiate receptors[19–22], whereas sites described as deficient in opiate receptors, such as the cerebral cortex, cerebellum and the medulla, showed no evidence of *N*-demethylation. Therefore there is coincidence between the sites of this biotransformation and the presence of opiate receptors, which supports the hypothesis that the receptor-mediated mechanism of opiate action involves *N*-demethylation. Further support for this concept was sought in experiment 5, in which the presence of the narcotic antagonist naloxone was expected to affect the demethylation of morphine in the CNS. But neither in the CNS nor in the liver were there any clear changes in morphine *N*-demethylation. These negative results suggest that either the reaction is not pharmacologically significant, or that the antagonist interferes with the chain of events subsequent to *N*-demethylation of the agonist. Alternatively, the antagonist may affect either the time scale or quantitative aspects of *N*-demethylation in a manner which does not become evident in the conditions we used.

We have previously utilised a double isotope procedure with *N*-$^{14}CH_3$-morphine and ^{3}H-naloxone in an effort to demonstrate displacement of the agonist by the antagonist in specific central sites[23]. We knew that *N*-demethylation might distort the results and performed a reverse isotope experiment in which 6-^{3}H-morphine and ^{14}C-naloxone were used. There was no evidence of any change in isotope ratio, and we assumed that *N*-demethylation of morphine does not proceed to a sufficient degree in the CNS to be detectable by these methods. These experiments, however, were carried out with sub-pharmacological doses of morphine. In subsequent experiments using pharmacological doses of *N*-$^{14}CH_3$-morphine and ^{3}H-naloxone we observed greater ratios of ^{3}H to ^{14}C in the medial thalamus and hypothalamus and interpreted these changes as indicative of displacement of morphine by the antagonist in these tissues. In view of these results, these interpretations must be revised since they were due to *N*-demethylation of the morphine and loss of the ^{14}C fragment from the tissue. The extent of this biotransformation is comparable with the 'displacement' observed. Displacement of agonist by antagonist from receptors *in vivo* is still presumed to occur, but in amounts not detectable by our procedures.

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Immunocytochemical evidence for the presence of arginine vasotocin in the rat pineal gland

THE discovery that the pineal gland contained melatonin[1] provided the stimulus for a multidisciplinary approach to the study of this organ. Considerable evidence suggests that this gland affects reproductive physiology. For example, it contains biologically active polypeptides. One of the first of these to be identified was arginine vasotocin (AVT), initially isolated from extracts of bovine pineal gland[2], and later identified chemically and found to have antigonadotropic properties[3,4]. There is physiological and radioimmunoassay evidence for the origin of AVT in the pineal gland. We now present immunocytochemical evidence for the presence of this polypeptide in the pineal gland.

AVT was conjugated to bovine serum albumin, and antiserum to the complex was prepared in adult male guinea pigs. The antiserum was absorbed with 1 mg of bovine serum albumin per ml of undiluted serum before use. Pineal glands and hypothalami from ten adult male rats were fixed in Bouin's solution and embedded in paraffin. Immunochemical staining was carried out with undiluted antiserum to AVT according to the procedure of Nakane and Pierce[7]. Controls similar to those described by Petrusz[8] were carried out to assure specificity of staining.

Because of the marked structural similarity of AVT, arginine vasopressin (AVP) and oxytocin, and because other investigators have claimed that the pineal gland contains AVP[6] and luteinising hormone releasing hormone (LHRH)[9], establishment of the specificity of the antiserum to AVT was important. The findings summarised in Table 1 strongly suggest that the antiserum to AVT was specific for AVT. Moreover, the supraoptic nucleus, the paraventricular nucleus and the median eminence (regions reputedly rich in AVP, oxytocin, and LHRH, respectively) did not stain with antiserum to AVT, substantiating the immunocytochemical specificity of the antiserum to AVT.

Antiserum to AVT immunochemically stained a distinct population of cells in the rat pineal gland (Fig. 1). These cells were distributed diffusely throughout the gland. They were very irregular in form with extensive perivascular and intercellular processes. Cytoplasmic processes were frequently cupped around neighbouring cells. Most parenchymal cells were regular in outline and were identified as cells clasically described as pinealocytes or chief cells. Their characteristic pale nuclei with infolded nuclear membranes and many nucleoli were in marked contrast to the intensely staining, elongate nuclei of the immunochemically stained cells. The pronounced staining of the nuclei of the presumptive AVT cells was interesting because this was not normally observed after immunocytochemical staining of cells containing polypeptides.

Fig. 1 Light micrograph of a section from a rat pineal gland immunochemically stained with antiserum to AVT. The darkly staining reaction product of the Nakane–Pierce technique defines the presumptive AVT cells.

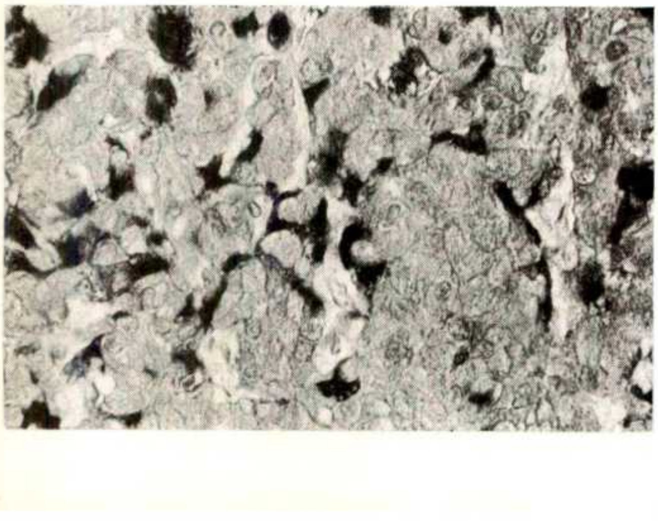

Table 1 Immunological specificity of antiserum to AVT

	Anti-AVT	Anti-AVT + AVT	Anti-AVT + AVP	Anti-AVT + OT	Anti-AVT + LHRH
Pineal gland	+	−	+	+	+

Immunological specificity was tested by absorbing undiluted antiserum to AVT with 0.2 mg of AVT, AVP, OT, or LHRH, before immunochemical staining of rat pineal sections.

These findings add further evidence for the presence of AVT in the pineal gland. Whether the immunocytochemically stained cells of this study represent a second line of pinealocytes or are glial in origin, and whether they synthesise or store AVT has yet to be determined.

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Functional roles of luminal sodium and potassium in water transport across desert scorpion ileum

IN terrestrial insects the rectum is a major site of fluid absorption and is, therefore, important in the physiological control of water balance. Water absorption in the insect rectum occurs against osmotic gradients greater than 300 mosmol l^{-1} and apparently in the absence of net ion movements from lumen to haemolymph[1,2]. Evidence suggests that net water flow from the rectal lumen to haemolymph results from increases in intercellular space osmotic pressure due to the transport across lateral cell membranes of recyclable intracellular solutes[3,4]. Little is known about the mechanism of water transport in the insect gut anterior to the rectum nor about gastrointestinal processes involved in water regulation in other terrestrial arthropods. Scorpions are ideal organisms for measurements of arthropod ileal transport because this part of the gut extends nearly the entire length of the tail and can be isolated for *in vitro* perfusion. We present here preliminary information on a sodium-dependent, potassium-inhibited water transport mechanism in the ileum of the desert scorpion *Hadrurus arizonensis*.

Scorpions were anaesthetised lightly with chloroform, and the entire ileum (2 cm) was removed and placed in a saline medium. The ionic composition and osmotic pressure of the medium were based on data obtained from flame photometry, chloride titration and osmometry of haemolymph samples, as well as on values reported from other scorpion species in the literature[5-7]. This scorpion saline had an osmotic pressure

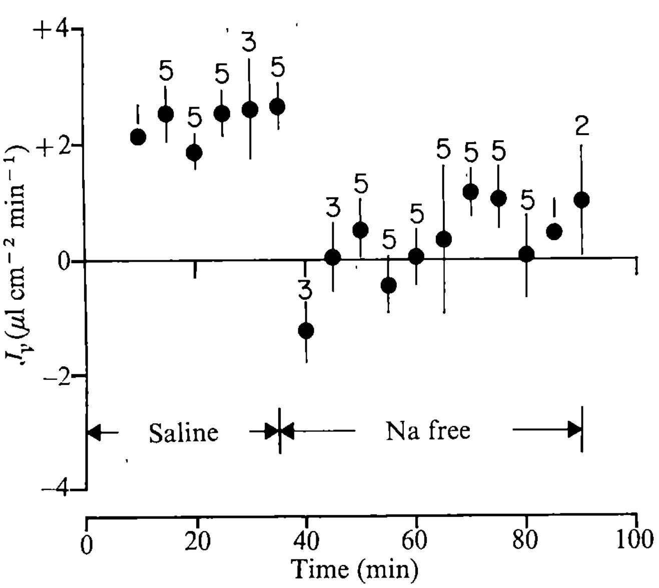

Fig. 1 Effect of sodium-free perfusate on net water flux across scorpion ileum. Ilea were first exposed to normal saline on the luminal surface for 35 min and were subsequently perfused for 55 min with medium lacking sodium. Normal saline was present in the serosal bath at all times. A positive net water flux indicates movement from mucosa to serosa. Circles represent mean flux values, vertical lines are ±1 s.e.m. and numbers signify sample size.

of 550 mosmol l^{-1}, a *p*H of 7.4, and consisted of the following ion concentrations in mM: Na, 281.5; K, 8.0; Ca, 10.0; Mg, 20.0; Cl, 260.0; HCO_3, 1.5; SO_4, 44. When incubation media of altered ionic composition were used, choline chloride was substituted for NaCl and KCl, and $KHCO_3$ for $NaHCO_3$; KCl concentrations were increased at the expense of those of NaCl (Na_2SO_4 replacing the altered NaCl), and raffinose was added to maintain approximately equal osmotic pressures on both sides of the epithelium. Ionic concentrations of normal ileal fluid were also measured by flame photometry after collection and centrifugation in haematocrit capillary tubes.

Each end of the isolated ileum was mounted on epoxy-coated 18-gauge stainless steel needles with surgical thread, immersed in 3 ml of scorpion medium in a lucite chamber, and perfused with saline by means of a polystaltic pump at a flow rate of about 125 µl min^{-1}. Net water transport (J_v) was measured by the procedures developed for perfused kidney nephrons[8] where the change in perfusate ^{131}I-albumen concentration (Squibb), after passage through the tubular preparation, provides information about the extent and direction of water flow. The perfusion rate (V_0, µl min^{-1}) was calculated as

$$V_0 = {}^{131}I_{tot}/[{}^{131}I_0]t$$

where [$^{131}I_0$] is the specific activity in the perfusion fluid (d.p.m. µl^{-1}); $^{131}I_{tot}$ the total amount of radioactivity in a sample of ileal fluid collected after passage through the gut (d.p.m.), and t the duration of the collection period (min). The rate of collection (V_f, µl min^{-1}) was estimated directly from the volume, determined gravimetrically to the nearest 0.1 mg, and duration of each collection. The net water transport rate J_v (µl cm^{-2} min^{-1}) was calculated as

$$J_v = (V_0 - V_f)/A$$

where A represents the estimated mucosal area (cm^2) determined by considering the ileum as a cylinder. A positive value of J_v reflects net movement of water from mucosa to serosa. Ilea were typically perfused with ^{131}I-albumen-labelled medium for the first 35 min (control interval), followed by perfusion for 45–55 min with medium of altered ionic composition containing the radioactive marker. Collections were made every 5 min. The serosal bath was periodically sampled for radioactivity to test for leaks. ^{131}I-albumen samples were counted in a Beckman LS-230 liquid scintillation spectrometer using a Triton X-100/toluene/Omniflour (New England Nuclear) scintillation cocktail.

Figure 1 shows that when the transmural osmotic gradient was zero (determined by measuring directly the osmotic pressures of mucosal and serosal solutions), J_v in scorpion medium was $+2.40 \pm 0.18$ µl cm^{-2} min^{-1} (mean ± s.e.m.; n = 24). That this net movement of water was energy dependent was shown in another experiment where the saline bathing both ileal surfaces contained 1 mM NaCN and 1 mM iodoacetate. Net flow in these conditions was reduced to +0.14±

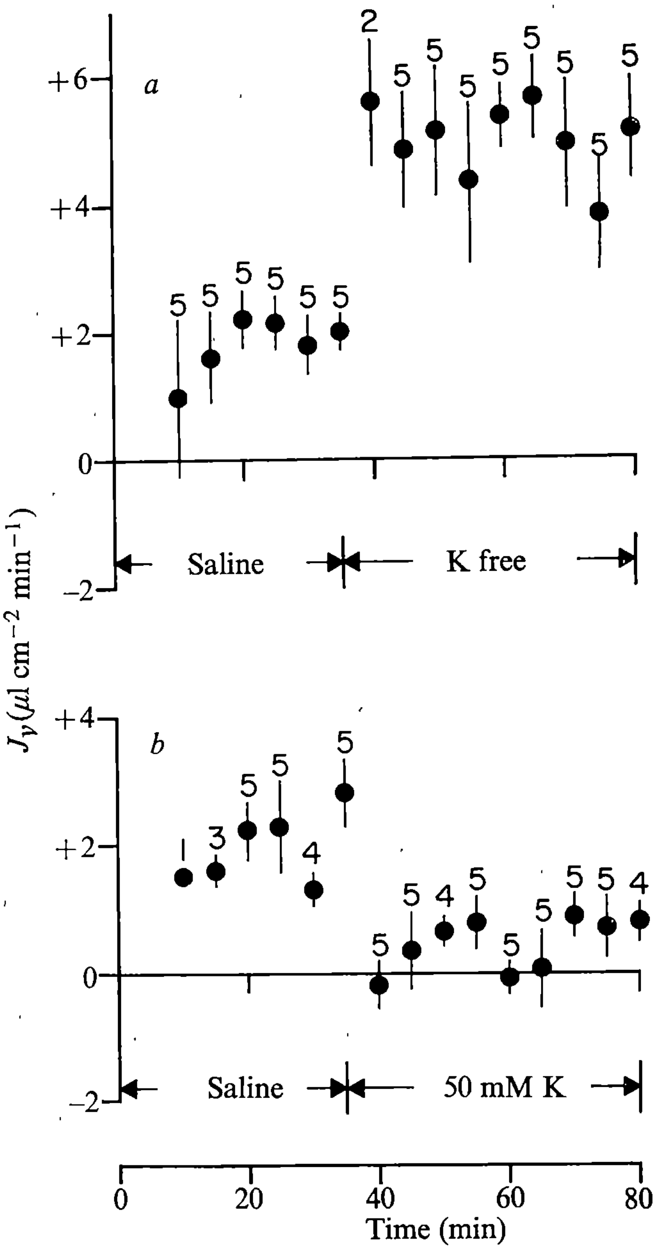

Fig. 2 *a*, Effect of potassium-free perfusate on net water flux across scorpion ileum. Ilea were first exposed to normal saline on the luminal surface for 35 min and were subsequently perfused for 45 min with medium lacking potassium. Normal saline was present in the serosal bath at all times. A positive net flux indicates water movement from mucosa to serosa. Symbols are as described in Fig. 1. *b*, Effect of increasing the potassium concentration of the perfusate from 8.0 mM (normal condition) to 50 mM on net water flux across scorpion ileum. Ilea were first exposed to normal saline on the luminal surface for 35 min and were subsequently perfused for 45 min with medium containing 50 mM potassium and normal sodium. Normal saline was present in the serosal bath at all times. A positive net flux indicates water movement from mucosa to serosa. Symbols are as described in Fig. 1.

Table 1 Effects of starvation and dehydration of *Hadrurus arizonensis* on sodium and potassium concentrations of centrifuged ileal contents

Ions in ileal fluid	Freshly fed*	Starved and partially dehydrated†	Starved and severely dehydrated‡
Na	123.3±17.8 (5)	177.0±38.6 (3)	468.0±112.4 (7)
K	274.9±41.5 (5)	131.8±58.1 (3)	83.2± 24.3 (7)

Ion concentrations are expressed in mM (mean±s.e.m.) and numbers in parentheses represent sample sizes.

*Scorpions fed crickets the day before sampling.

†Scorpions maintained without food at 25 °C and at about 30% relative humidity for 2 weeks before sampling.

‡Scorpions maintained without food at 35 °C and about 30% relative humidity for 4 weeks before sampling.

0.24 $\mu l\ cm^{-2}\ min^{-1}$ ($n=36$), which is not significantly different from zero ($P>0.05$). Figure 1 also indicates that when ilea were perfused with Na-free medium (normal medium on serosal side), the net flux of water from mucosa to serosa was eliminated ($J_v = +0.28\pm0.22$ $\mu l\ cm^{-2}\ min^{-1}$; $n=44$; $0.20>P>0.10$). An analysis of variance test comparing J_v for the control group and net water flux for the Na-free preparations showed the two groups to be significantly different ($P<0.005$).

When potassium ion was eliminated from the saline perfusate and sodium ion remained normal (281.5 mM) net water flux from the mucosal to serosal side of the preparation increased significantly ($P<0.005$) above the control value of $+1.79\pm0.26$ ($n=30$) to 4.92 ± 0.29 ($n=42$) $\mu l\ cm^{-2}\ min^{-1}$ (Fig. 2, top). An increase in luminal potassium concentration from 8.0 to 50.0 mM at constant luminal sodium concentration (281.5 mM) resulted in a significant decrease ($P<0.005$) in mucosal to serosal J_v from a control value of $+1.95\pm0.23$ ($n=23$) to $+0.43\pm0.14$ ($n=43$) $\mu l\ cm^{-2}\ min^{-1}$ (Fig. 2, bottom). The latter value is still significantly greater than zero ($P<0.01$). Control J_v values from the above three experiments, compared using analysis of variance, were not significantly different ($P>0.05$).

Scorpions that had eaten the day before sampling illustrated ileal sodium and potassium concentrations of 123.3±17.9 mM ($n=5$) and 274.9±41.5 mM ($n=5$), respectively (Table 1). Starving and dehydrating animals at temperatures up to 35 °C and for periods up to 4 weeks significantly increased the ileal sodium concentration ($n=15$; $P<0.05$) and significantly decreased the potassium concentration ($n=15$; $P<0.01$). In addition, the ratio of sodium to potassium was reversed. In freshly fed scorpions, Na/K was 0.46, whereas in starved and dehydrated scorpions Na/K was 5.6 (an ionic ratio that produced a net flux of water from lumen to blood in Fig. 2, bottom).

This is the first detailed study of a water transport mechanism in the ileum of a terrestrial arthropod, although Wigglesworth and Ramsay have suggested[9,10] that some water absorption occurs in this portion of the insect gut. Our data suggest that the ileal absorption of water in the desert scorpion occurs through a sodium-dependent, potassium-inhibited transport process. This mechanism is clearly adaptive as dehydration and starvation lead to intraluminal sodium and potassium concentrations which favour the uptake of water. In freshly fed animals excessive hydration could be reduced by the inhibitory interaction of potassium ion on the sodium-dependent water movement. Water balance in these organisms may depend on the relative sodium/potassium ratio present in the ileum. This will require further study.

The anatomical location of the inhibitory effect of potassium on sodium-dependent water movement remains unclear. The scorpion ileum, in contrast to that of insects, is unchitinised and has a tall columnar epithelium[11]. Therefore, a standing osmotic gradient[12-14] in the long intercellular spaces created by an electrogenic, energy-requiring lateral membrane cation pump could satisfactorily generate the transmural water fluxes observed. The rate of sodium efflux by the cation pump would be a function of the intracellular sodium concentration, which, in turn, would depend on the luminal concentration of this ion. Potassium ion, derived from the ileal lumen could compete with sodium for attachment to this lateral membrane cation pump and convert its electrogenicity into a simple K–K exchange involving no net ionic fluxes. A reduction or elimination of net transmural water flow would then result from a decrease in the intercellular standing osmotic gradient. In contrast, competition between sodium and potassium for a shared carrier process in the apical membrane, followed by active sodium efflux across the lateral membrane could equally well account for the effects of ions on water flow described here. Further studies of ion fluxes in the scorpion ileum are clearly needed to establish more thoroughly their role in transmural water movements.

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Fast block to polyspermy in sea urchin eggs is electrically mediated

THE prevention of polyspermy in sea urchin eggs is accomplished in two steps: a "fast" block, occurring within a few seconds of the entry of the fertilising sperm, and a "slow" block, associated with the cortical reaction (compare refs 1–4 with 5 and 6). Results presented here confirm the existence of the fast block and demonstrate that it is caused by the electrical depolarisation of the egg plasma membrane that accompanies the entrance of the fertilising sperm.

Eggs of *Strongylocentrotus purpuratus* were hand centrifuged and agitated mechanically with a jet of seawater, to remove their jelly coats. Some of the dejellied eggs adhered to the bottom of Falcon plastic Petri dishes, such that a microelectrode could be inserted. All experiments involved eggs with a stable resting potential of greater than −60 mV (Table 1). These values of resting potential are close to that calculated from independent ion tracer flux studies done on these eggs (my work in preparation with K. R. Robinson). A single microelectrode was used for both recording voltage and passing current, by means of a conventional bridge circuit. Experiments were done at 15 °C, in natural or artificial seawater, Tris-buffered at *p*H 8. In all experiments, the concentration was about 10^6 sperm per ml, which is within the range that can produce polyspermy in some batches of eggs, but not in most. Polyspermy was determined by observation of first cleavage: monospermic eggs divide into two equal cells, whereas polyspermic eggs

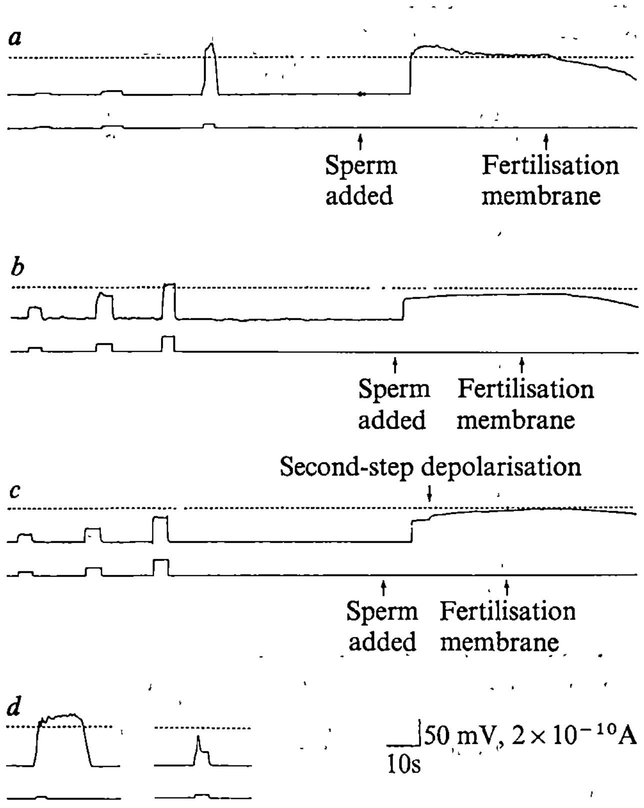

Fig. 1 Action potentials and activation potentials in *S. purpuratus*. The top trace is voltage against time; the bottom trace is current against time. The dotted line indicates 0 mV. Before addition of sperm, each egg membrane was tested for electrical excitability by applying a series of small depolarising current pulses. *a*, Egg with regenerative response to current pulse; monospermic activation potential. *b*, Egg with non-regenerative response; monospermic activation potential. *c*, Egg with non-regenerative response; polyspermic activation potential. *d*, Other examples of the regenerative response; a strong response outlasting the stimulus, a weak response.

cleave abnormally, dispermic eggs usually producing three or four cells at once[7,8].

Figure 1 shows intracellular electrical records from three eggs before, during and after addition of sperm. The fast depolarisation after the addition of sperm has been called the "activation potential"[9-15]. Within 3–30 s after the introduction of sperm near the egg, the egg membrane depolarises to a plateau at −30–+20 mV, and then after about 1 min begins to repolarise.

An interesting correlation was found between the plateau level of the activation potential (measured 3 s after its rise) and the occurrence of polyspermy (Table 1). Eggs with activation potentials which reached a plateau more positive than 0 mV were never polyspermic (none out of eight cases) and those which reached a plateau less positive than −10 mV were sometimes polyspermic (seven out of 13 cases). These results suggest that the entry of extra sperm is prevented by the more positive-going activation potential.

Figure 1*c* shows an activation potential in a polyspermic egg. About 5 s after the initial rise, there was a distinct second step depolarisation (see arrow). Eleven polyspermic activation potentials were observed (seven listed in Table 1, four not shown). All 11 showed this second step, and sometimes a third step. Ten monospermic activation potentials, as in Fig. 1*b*, were observed; of these, eight definitely did not show a second step. Two cases did show a second step; their nature is obscure. (In cases like that in Fig. 1*a*, with an oscillating plateau level, the steps could not be counted.) These results indicate that the second step depolarisation is associated with the entrance of a second sperm. The voltage at which this second step occurs is always more negative than −10 mV, suggesting that when the egg membrane is depolarised beyond −10 mV, sperm penetration is less probable.

Figure 2*a* shows that when current was applied to hold the unfertilised egg membrane at a potential more positive than +5 mV, sperm did not fertilise the egg. In spite of the presence of many active sperm adhering to the egg surface, no fertilisation membrane formed, and no electrical event resembling an activation potential occurred. In some experiments, the potential was held for more than 5 min, with repeated additions of sperm; but still no fertilisation occurred in the voltage-clamped eggs. (Surrounding eggs formed fertilisation membranes within 1 min of the first addition of sperm.) As soon as the current was turned off, fertilisation occurred: the egg depolarised, showed a typical activation potential, formed an apparently normal fertilisation membrane with the usual time course, and, if the activation potential was of the one-step form, first cleavage was normal, indicating that only one sperm had entered the egg. The same result was obtained with holding potentials ranging from +5 to +130 mV. In other experiments, the potential was held for about 10 min, without addition of fresh sperm, after which most sperm had become less active and incapable of fertilising eggs. When the current was turned off, the egg membrane returned to its original resting potential and showed the electrical properties characteristic of the unfertilised state. Morphologically, there was no sign of activation, and no development occurred. If more sperm were added, fertilisation and development proceeded in the usual way.

Fertilisation is prevented at +5 mV; but it occurs at −10 mV, as determined by the experiments reported in Table 2. The egg was depolarised enough to block fertilisation, and sperm were added. After 3–5 min, the voltage was lowered to a point where fertilisation occurred. Each experiment sets an upper and a lower limit on the threshold voltage for suppressing sperm entry. This is between +5 and −10 mV; at intermediate potentials, entry of sperm may depend on their concentration and activity, and on individual variation among eggs. This threshold is consistent with the following observations on activation potentials. (1) Eggs whose activation potential plateau (measured 3 s after the initial rise) was more positive than 0 mV, are

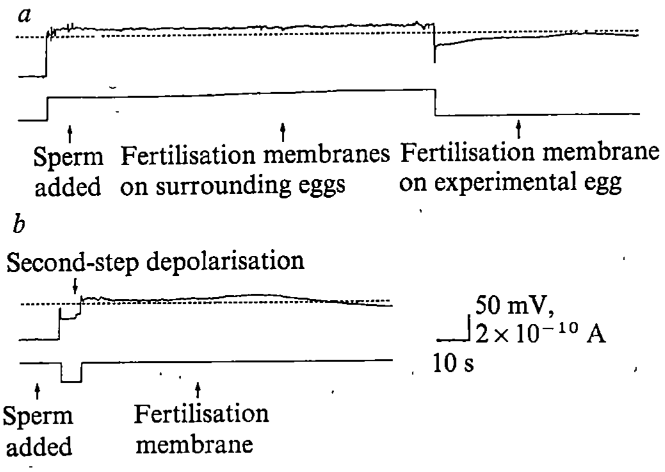

Fig. 2 Demonstration of potential dependence of sperm entrance. The top trace is voltage against time; the bottom trace is current against time. The dotted line indicates 0 mV. *a*, Suppression of fertilisation by positive holding potential. *b*, Induction of polyspermy by negative holding potential. In (*a*) the amount of current required to maintain the holding potential increased about 10% during the first 1–2 min after the current was turned on. This increase was seen even if sperm were not added. In some experiments, sperm were not added until this initial adaptation of the egg membrane was complete. The addition of sperm did not cause any change in the amount of current required to maintain the potential.

Table 1 Activation potentials for monospermic and polyspermic eggs

Egg	Resting potential (mV)	Regenerative response	Activation potential plateau (mV)	First cleavage	No. of steps in rise of activation potential	Voltage at second step (mV)
A	−75	Strong	+23	M	—	—
B	−69	Strong	+17	M	—	—
C	−68	Strong	+15	M	—	—
D	−76	Strong	+14	M	—	—
E	−77	Strong	+12	M	—	—
F	−74	Strong	+10	M	—	—
G	−71	Strong	+ 6	M	—	—
H	−71	Weak	0	M	—	—
I	−71	Weak	−12	P	2	− 9
J	−74	NT	−13	M	1	—
K	−68	Weak	−14	M	1	—
L	−75	Weak	−15	M	1	—
M	−56	NT	−17	P	2	−15
N	−60	None	−19	M	1	—
O	−63	None	−20	M	1	—
P	−79	Weak	−20	P	2	−14
Q	−71	Weak	−22	P	2	−22
R	−64	None	−23	P	2	−22
S	−70	Weak	−26	M	2	−27
T	−65	None	−29	P	2	−26
U	−78	NT	−35	P	3	−35, −24

The activation potential plateau was measured 3 s after the initial rise. Eggs were classified as monospermic (M) if first cleavage produced two equal cells; otherwise, they were classified as polyspermic (P). The regenerative response of the unfertilised egg was classified as strong if in response to a 5-s current pulse of 3×10^{-11} A, the membrane maintained a positive potential for the duration of the pulse. None means that the response was completely, or almost completely, non-regenerative. Weak means that there was some regenerative response, but it was not strong. NT, Not tested.

always monospermic. (2) The entrance of the second sperm in polyspermic eggs occurred at a voltage more negative than −10 mV.

Application of current to bring the plateau of the activation potential to a more negative level facilitated entry of additional sperm (Fig. 2*b*). After the activation potential began, current was applied to hold the potential at −30 mV. A second-step depolarisation occurred, then the current was turned off. Observation of first cleavage showed that this egg was polyspermic, whereas 98% of the surrounding eggs in the dish were monospermic. This experiment was repeated seven times, with holding potentials of −30 ± 5 mV. In all cases, experimental eggs were polyspermic and surrounding eggs were monospermic.

These experiments demonstrate that the electrical depolarisation occurring at fertilisation constitutes a fast block to polyspermy. It was frequently observed that when the electrically monitored egg was polyspermic, other eggs in the dish were also polyspermic (except in cases where current was applied to make an egg polyspermic). Therefore, the lack of a fast block to polyspermy is a property of a batch of eggs. Loss of the capacity to block polyspermy might be a symptom of egg deterioration, either in the adult, or after removal from the adult.

The rise of the activation potential can occur within 3 s of insemination. Part of this time is necessary for the sperm to swim to the egg surface and to attach to the vitelline membrane. Only then does the sperm fuse with the egg plasma membrane. Therefore, it can be concluded that the fast block to polyspermy is established in less than 3 s after fusion of the fertilising sperm. Quite possibly, the rise of the activation potential is a direct consequence of the disturbance of the egg membrane by sperm fusion. If this is the case, it follows from the rate of rise of the activation potential that the fast block to polyspermy is established in less than 1 s after fusion. (The rise from resting level to −10 mV takes between 0.1 and 1 s.) The membrane remains in the depolarised state for about 1 min, after which the cortical reaction has occurred, thus providing a permanent block to polyspermy.

In each example in Fig. 1, a series of small depolarising current pulses was applied to the egg before sperm were added. In the case shown in Fig. 1*a*, the stimulus pulse induced a regenerative response of the egg membrane. This response showed a threshold at about −50 mV, and sometimes outlasted the stimulus (Fig. 1*d*); it has been observed in other echinoderm oocytes and eggs[15–17] and has been studied in sea urchin eggs by K. Takahashi (personal communication). It is an action potential of the sort seen in nerve and muscle (as distinct from the activation potential). Figure 1*b* and *c* illustrates eggs which did not show a regenerative response to depolarisation. These eggs showed less positive-going activation potentials after sperm addition, compared with the egg in Fig. 1*a*. This relationship between the presence of the regenerative response and the height of the activation potential is also shown in Table 1. The correlation indicated that the activation potential is caused partly by a regenerative response of the egg membrane. The data in Table 1 suggest that the sperm fusion depolarises the egg to a level of about −30

Table 2 Voltage threshold for suppression of fertilisation

Egg	Suppressing potential (mV)	Non-suppressing potential (mV)
A	+6	−6
B	+3	−11
C	+2	−10
D	+1	−10
E	+5	−7
F	+3	−12
G	+5	−6
H	+6	−5
I	—	−2

The egg membrane was held at the suppressing potential and sperm were added. About 1 min later, all the surrounding eggs had formed fertilisation membranes. 3–5 min after addition of sperm, the potential was reduced to the non-suppressing level: within 1–2 min, the experimental egg formed a fertilisation membrane. After removal of the electrode, balance of the WPI bridge circuit was tested by applying a current pulse of the same magnitude used in the experiment. If the imbalance was greater than 2 mV, data were not used. Error associated with the single microelectrode technique was small, because of the small currents used ($0.3\text{–}3 \times 10^{-10}$ A), and the high input resistances of the cells (200–2,000 MΩ, depending on the degree of regenerativeness of the egg's response), compared with the resistances of the microelectrodes (40–80 MΩ).

to −20 mV. Then, in eggs which have the capacity to produce a regenerative response, the sperm-initiated depolarisation is amplified, bringing the egg membrane potential to a level which excludes additional sperm.

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Translation of RNA from unfertilised sea urchin eggs does not require methylation and is inhibited by 7-methylguanosine-5′-monophosphate

AT fertilisation, the rate of protein synthesis in the sea urchin embryo becomes several times greater than that observed in unfertilised eggs[1–4]. This increase does not require the synthesis of messenger RNA (mRNA), since it occurs both in the presence of actinomycin D (ref. 5) and in enucleated parthenogenetically activated egg fragments[6]. These observations led Spirin[7] to postulate the existence of preformed "maternal" mRNA in the eggs. Recent experiments have directly demonstrated the presence of mRNA in sea urchin egg cytoplasm by isolating it from postribosomal cytoplasmic fraction and translating it in a heterologous cell-free system[8].

The mechanism(s) responsible for the increased rate of protein synthesis at fertilisation is still not understood. Some investigators have attributed this increased rate to the removal of an inhibitor of protein synthesis from the egg ribosomes[9,10]. Clegg and Denny[11], however, have reported that ribosomes isolated from unfertilised eggs translate rabbit globin mRNA as efficiently as ribosomes isolated from zygotes when supplied with ascites cell postribosomal supernatant and reticulocyte initiation factors. These authors concluded that the low protein synthetic activity in unfertilised eggs is not attributable to the presence of inhibited ribosomes[11].

Activation of mRNA by adenylation may also be important in the increased rate of protein synthesis[12,13]. The mRNAs coding for histones are never adenylated[14], however, but make up a considerable proportion of maternal mRNA[8,15] Adenylation thus does not have any role in the translational activation of this class of mRNAs, although it may be involved in increasing the stability of other mRNAs[16]. In this paper we discuss two other mechanisms for the activation of maternal mRNA which involve the methylation of the 5′-terminal nucleotide or the addition of 5′-terminal 7-methylguanosine.

The 5′ termini of many viral and cellular mRNAs have been shown to contain a 7-methylguanosine residue linked, by way of a triphosphate, to the 5′-hydroxyl of the adjacent nucleotide ($m^7G^{5'}ppp^{5'}Xp$) (ref. 17). Removal of the 5′-terminal $m^7G^{5'}p$ from globin mRNA prevents its translation[18]. Moreover, Both *et al.*[19] have shown that the presence of the methyl group in the 5′-terminal guanosine is required for translation of some viral RNAs. These RNAs can be obtained without a methyl group in the 5′-terminal guanosine by synthesising them *in vitro* in the presence of an inhibitor of methylation, S-adenosylhomocysteine (SAH)[20]. Wheat germ extracts can methylate these RNAs specifically to yield the structure $m^7G^{5'}ppp^{5'}G$ in the case of reovirus RNA, which can then be translated[19]. In the presence of SAH, however, the RNA is not methylated and cannot be translated[19,20]. Both *et al.*[19] considered the possibility that methylation may be involved in the activation of maternal mRNA at fertilisation.

The assay used so far to translate maternal mRNA could not discriminate between mRNAs methylated in the 5′-terminal

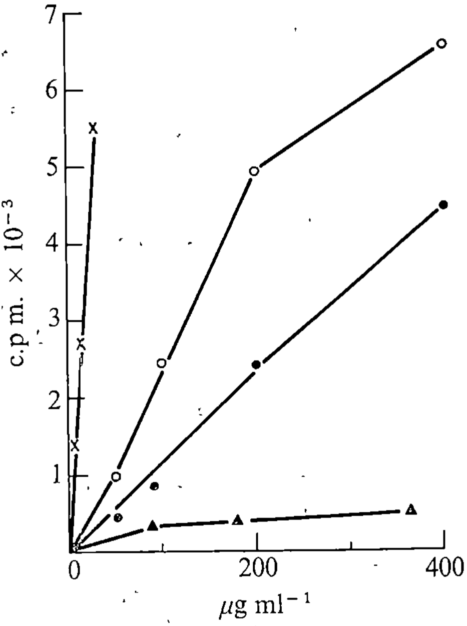

Fig. 1 Stimulation of protein synthesis in a wheat germ cell-free system by RNA isolated from unfertilised sea urchin eggs. Eggs were collected from *Lytechinus pictus* by injection of KCl and washed as described previously[8]. About 10 ml of pelleted eggs were homogenised at 0 °C with 10 strokes of a loose fitting Dounce homogeniser in 35 ml of a buffer containing 200 mM NaCl, 5 mM magnesium acetate, and 20 mM Tris, *p*H 7.6. A post-mitochondrial fraction was obtained by centrifugation at 17,000*g* for 15 min. This fraction was adjusted to contain 1% sodium dodecyl sulphate and 10 mM EDTA, then warmed to room temperature and deproteinised with phenol–chloroform–isoamyl alcohol (25:24:1) (ref. 28). RNA was recovered by ethanol precipitation and separated into poly(A)+ and poly(A)− fractions by chromatography on oligo(dT)–cellulose[22]. A portion of the poly(A)− RNA was further fractionated by sucrose gradient centrifugation. The RNA sedimenting in the 6–12S region of the gradient was recovered by ethanol precipitation. All RNA preparations were reprecipitated from ethanol three times before translation in a wheat germ cell-free system[21]. Each assay contained in 25 µl: 7.5 µl of wheat germ extract, 1.2 mM ATP, 0.25 mM GTP, 15 mM creatine phosphate, 0.34 units of creatine phosphokinase (Sigma), 22 mM HEPES–KOH buffer *p*H 7.1, 3 mM magnesium acetate, 0.1 M KCl, 2.6 mM DTT, 1.8 mM mercaptoethanol, 50 µM unlabelled amino acids minus lysine, and 2.5 µCi of ^{3}H-lysine (38 Ci mmol^{-1}). RNA was added to give the concentrations indicated. The incubation was carried out for 60 min at 24 °C and 5-µl samples were taken in duplicate for counting as described previously[21]. A background of 800 c.p.m. obtained in an incubation without added RNA was subtracted from each value. Nonspecific stimulation resulting from the addition of ribosomal RNA (rRNA) was monitored by the addition of 16S RNA isolated from *E. coli* ribosomal subunits by phenol extraction and sucrose gradient centrifugation. ×, Poly(A)+ (total); ○, poly(A)− (6–12S); ●, poly(A)− (total); ▲, *E. coli* 16S rRNA.

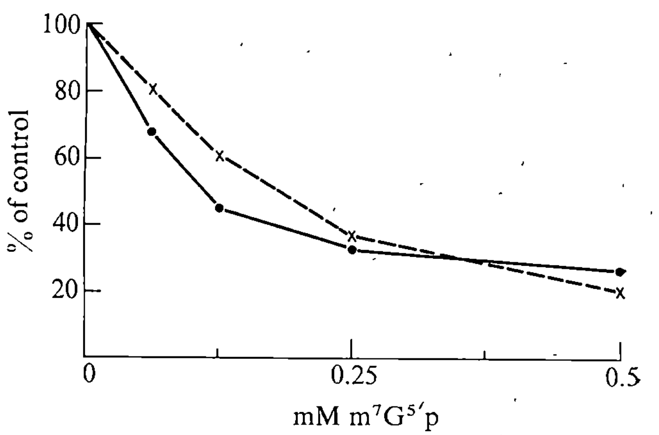

Fig. 2 Inhibition by $m^7G^{5'}p$ of the translation of poly(A)+ RNA isolated from unfertilised sea urchin eggs (●) and HeLa cells(×). The incubations were carried out as described in Fig. 1. Sea urchin egg poly(A)+ RNA was added to give 95 µg ml^{-1} and HeLa poly(A)+ RNA to give 36 µg ml^{-1}. Translation is expressed as a percentage of c.p.m. of incubations carried out in the absence of inhibitor. These values were 10,700 c.p.m. for sea urchin and 11,900 c.p.m. for HeLa RNA when corrected for the endogenous protein synthesis of the wheat germ extract.

guanosine and unmethylated mRNAs because of the endogenous methylating activity of the wheat germ extract already discussed. We have thus translated maternal mRNA in the presence of SAH to prevent its methylation during cell-free translation. In subsequent experiments we used a specific inhibitor of the translation of mRNAs with the 5′-terminus $m^7G^{5'}ppp$ (ref. 21) to show that all the maternal mRNAs translated in a wheat germ cell-free system already have this 5′-terminal sequence before fertilisation.

Eggs of *Lytechinus pictus* were homogenised and RNA prepared as described in the legend of Fig. 1. The RNA was fractionated into polyadenylated (poly(A)+) and non-polyadenylated RNA (poly(A)−) by chromatography on oligo(dT)-cellulose[22]. The poly(A)+ RNA stimulated protein synthesis 10–15 times over the endogenous level of the wheat germ extract. Somewhat greater stimulation is obtained with poly(A)+ RNA from HeLa cells (Fig. 2)[21]. The poly(A)− RNA stimulates protein synthesis much less than the poly(A)+ RNA as a result of the large amount of non-translatable ribosomal and transfer RNA in this fraction. This stimulation is, however, consistently higher than that obtained with *E. coli* 16S ribosomal RNA (Fig. 1). Better stimulation of protein synthesis is obtained by translating the fraction of poly(A)− RNA which sediments between 6S and 12S during sucrose gradient centrifugation (Fig. 1). This fraction is enriched for histone mRNA[23].

Translation of egg RNA is not affected by the presence of SAH (Table 1), indicating that methylation is not required for the translation of this RNA. On the other hand, addition of $m^7G^{5'}p$ inhibits translation of poly(A)+ sea urchin RNA to the same extent as poly(A)+ RNA from HeLa cells (Fig. 2). This latter RNA has been shown to have the 5′-terminal sequence $m^7G^{5'}ppp$ (ref. 24). We have recently shown that the translation of all HeLa poly(A)+ RNA species is equally inhibited by $m^7G^{5'}p$, by analysing their translation products synthesised in the presence of inhibitory concentrations of $m^7G^{5'}p$ (ref. 25). This nucleotide has no effect on the translation of satellite necrosis virus RNA in the wheat germ cell-free system[21], or of encephalomyocarditis virus RNA in an HeLa cell-free system[25]. Neither of these viral RNAs has the 5′-terminal sequence $m^7G^{5'}ppp$ (see ref. 25 for references).

Table 1 The effect of S-adenosylhomocysteine (SAH) on translation of sea urchin egg RNA

RNA	Concentration (µg ml^{-1})	^{3}H-lysine incorporation (c.p m.) Control	+0.32 mM SAH
Poly(A)+ (total)	95	9,455 (8.6×)*	10,589 (8.0×)
Poly(A)− (6–12S)	400	6,173 (3.9×)	6,958 (4.0×)

Experimental procedures are described in the legend of Fig. 1. The concentration of RNA is the final concentration in the assay mixtures. The concentration of SAH used (0.32 mM) inhibits the methylation of unmethylated reovirus RNA, which is required for its translation[19].

*Figures in parentheses indicate stimulation over endogenous incorporation (stimulation = (c p.m.+RNA)/(c.p.m.−RNA).

The experiments reported rule out methylation of mRNA as a significant mechanism for the increased rate of protein synthesis at fertilisation of sea urchin eggs. In addition, it is unlikely that a 5′-terminal guanosine, which already contains a methyl group, is added to egg mRNA during the incubation in the wheat germ cell-free system. The reovirus RNA synthesised *in vitro* consists of a mixture of molecules with the 5′ termini $m^7G^{5'}ppp^{5'}G$, $G^{5'}ppp5'G$, and pp5′G (ref. 20). Both *et al.*[20] have shown that only those molecules which already contain the $m^7G^{5'}ppp'G$ termini can bind to ribosomes and be translated by the wheat germ cell-free system in the presence of SAH. Therefore, the observation that egg mRNA is translated equally well in the presence or absence of SAH suggests that this RNA already contains the 5′-terminal $m^7G^{5'}ppp$ sequence found in most eukaryotic mRNAs. This is supported further by experiments showing that the translation of maternal poly(A)+ RNA is inhibited by $m^7G^{5'}p$ (Fig. 2).

We suggest that chemical modification of maternal mRNA does not have a significant role in the activation of protein synthesis at fertilisation. Maternal mRNA exists in the egg as ribonucleoprotein complexes[8]. The activation of protein synthesis at fertilisation may be a result of changes in the macromolecules associated with mRNA. Alternatively, the increased rate of protein synthesis may simply reflect the general metabolic activation of the zygote. A state of low respiratory activity occurs at the end of sea urchin oogenesis[26], although unfertilised eggs contain concentrations of ATP and GTP as great as embryos[27]. It has been proposed that cell-surface macromolecules are involved in maintaining the low metabolic state of unfertilised eggs[28]. Fertilisation leads to an increased rate of overall cellular metabolism, which can support an increased rate of protein synthesis. If this latter explanation is correct, it should be possible to isolate ribonucleoproteins from unfertilised eggs and translate them *in vitro*. We are at present carrying out further studies designed to test this possibility.

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RNA synthesis in fully-grown mouse oocytes

IT is now well documented for various species that the progress of early development depends to a large extent on materials synthesised and stored in the oocyte during oogenesis. A well studied example of this phenomenon is the synthesis and accumulation of both ribosomal and "informational" RNA in amphibian oocytes[1–3]. The extent of the contribution of oocyte RNA to early mammalian development is, however, not completely clear, since mouse embryos initiate rRNA synthesis at least as early as the four-cell stage of embryogenesis[4,5] and, unlike certain lower forms, the progress of early cleavage in the mouse is interfered with by inhibitors of RNA synthesis[6–8]. On the other hand, it is clear that growing mouse oocytes synthesise and store RNA[9–12], such that the ovulated egg contains approximately the same amount of RNA on a volume basis as the amphibian egg (calculated from refs 13 and 14).

Shortly after birth, the mouse ovary is populated with thousands of small oocytes arrested in late prophase ('dictyate') of meiosis. Commencement of oocyte growth is apparently regulated within the ovary, the number of oocytes entering the growth phase being a function of the size of the pool of non-growing oocytes[15,16]. The oocyte and its surrounding follicle grow coordinately, progressing through a series of definable morphological stages[17]. The oocyte completes its growth in the adult mouse before the formation of the follicular antrum; consequently, the

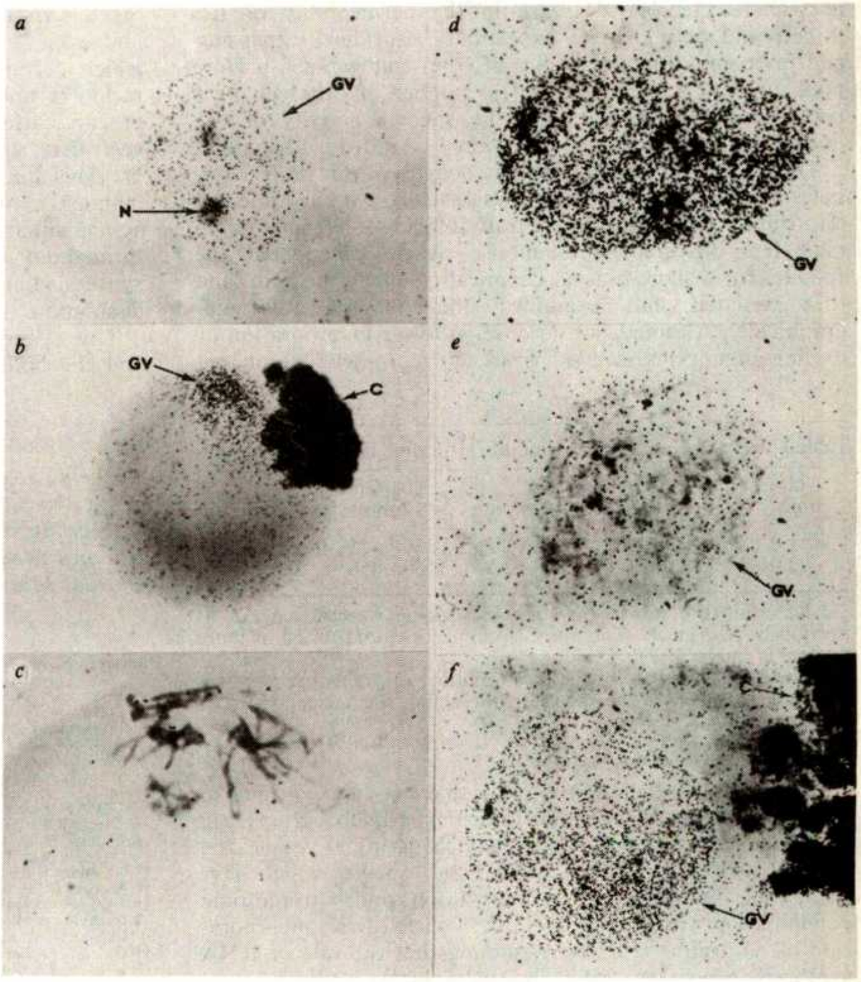

Fig. 1 Autoradiographs of mouse oocytes isolated from antral follicles micro-injected with ^{3}H-uridine or of mouse oocytes cultured *in vitro* with and without cumulus cells in the presence of ^{3}H-uridine. *a–c*, Follicles were dissected manually from the ovaries of adult (8–12 weeks of age), randomly bred, female Swiss mice (CD-1, Charles River Laboratories) and maintained in a chemically-defined culture medium[24]. The size of individual follicles was measured with an ocular micrometer attached to an inverted phase microscope. Each follicle was transferred, together with a small amount of medium, to a depression in an agar plate using a glass suction micropipette. A glass puncture micropipette attached to a Sensauer micromanipulator was used to inject approximately 1.0 nl of ^{3}H-uridine (10 mCi ml^{-1}, 26.2 Ci mmol^{-1}, New England Nuclear), made-up in culture medium, into each follicle. Follicles were then transferred through two washes of medium and cultured for 1 h at 37° C in an humidified atmosphere of 5% CO_2 in air. The entire injection and wash procedure required approximately 1 min for each follicle treated. At the end of the culture period, follicles were physically disrupted individually and the oocytes were placed on albumin-coated slides, cover slipped, and fixed by drawing a solution of alcohol–acetic acid (3 : 1 by volume) through. The slides were then frozen, cover slips flickedoff, and the slides washed and coated with Kodak NTB 2 liquid emulsion. Slides were stored in the dark at 4 °C for 5 d and were developed using standard procedures. In some cases preparations were stained with Giemsa (Harleco No. 620) at a 1 : 50 dilution with 0.1 M phosphate buffer, *p*H 6.8, for 30 min and/or treated with ribonuclease (0.1%, Sigma) for 4 h at 37 °C. Light microscopy was performed using a Zeiss Photomicroscope II. *a*, Oocyte from antral follicle, approximately 500 μm in diameter; *b*, Oocyte from antral follicle, approximately 450 μm in diameter, with adhering cumulus cells; *c*, Oocyte from antral follicle, approximately 500 μm in diameter, which has undergone germinal vesicle breakdown and chromosome condensation. *d–f*, Oocytes were obtained by puncturing ovaries from adult Swiss mice[25]. Oocytes containing an intact germinal vesicle, freed of or surrounded by cumulus cells, were collected using a mouth-operated micropipette and washed in culture medium. Cell culture was carried out in embryological watch glasses in medium containing ^{3}H-uridine (50 μCi ml^{-1}, 26.2 Ci mmol^{-1}, New England Nuclear) under paraffin oil at 37 °C in a humidified atmosphere of 5% CO_2 in air. Oocytes were cultured for 2 h, collected and washed, and those cultured in the presence of cumulus cells were denuded by repeated pipetting. The oocytes were then treated for autoradiography as above. *d*, Oocyte cultured in the presence of cumulus cells; *e*, Oocyte cultured in the absence of cumulus cells; *f*, Oocyte cultured in the presence of cumulus cells, with adhering cumulus cells. GV, germinal vesicle; N, nucleolus; C, cumulus cells.

majority of follicle growth occurs after the oocyte has stopped growing[18]. This report shows that mouse oocytes continue to synthesise RNA even during the period of follicular antrum formation when oocyte growth has ceased, a finding which differs with the current view that RNA synthesis in mouse oocytes is inhibited at the time of, and subsequent to, antrum formation[9,11].

We find that when ^{3}H-uridine is administered intraperitoneally to adult mice, and its incorporation into oocyte RNA is evaluated soon after by autoradiography of ovarian sections, there is a positive correlation between oocyte size and uridine incorporation within the oocyte's nucleus (germinal vesicle) in follicles lacking an antrum. Those follicles containing an incipient or highly developed antrum display virtually no grains over the oocyte's germinal vesicle or nucleolus. These observations are essentially identical to those of several other investigators and have led previous workers to conclude that at the time of antrum formation RNA synthesis within the oocyte is suppressed[9,11]. This means that for a period of several days, while the follicle undergoes tremendous growth, the fully-grown oocyte possessing an intact germinal vesicle and nucleolus is quiescent with respect to RNA synthesis. Alternatively, these observations could reflect, not the absence of RNA synthesis in oocytes of antral follicles, but rather, a precursor pool of UTP of such low specific activity that oocyte RNA synthesis goes undetected. A decrease in the permeability of the follicle and/or oocyte to exogenous ^{3}H-uridine, expansion and/or compartmentalisation of the endogenous uridine pool of the oocyte, or any of a number of other changes could lead to a decrease in the specific activity of the oocyte's UTP pool at about the time of antrum formation.

To explore the possibility that oocytes within antral follicles actually continue to synthesise RNA, we have injected ^{3}H-uridine directly into antral follicles (450-500 µm in diameter) dissected from mouse ovaries, incubated the follicles for a short time in chemically defined medium, and then isolated and examined the oocytes autoradiographically. Although we do observe lower incorporation of uridine in oocytes isolated from antral follicles compared with preantral follicles, it is clear that oocytes continue to synthesise RNA throughout follicular growth; grains are found concentrated within the oocyte's germinal vesicle and nucleolus (Fig. 1). On the other hand, oocytes which have undergone germinal vesicle dissolution and chromosome condensation within the follicle show virtually no incorporation of ^{3}H-uridine (Fig. 1). Assuming that the rate of RNA synthesis in oocytes within isolated follicles is the same as that *in vivo*, these results suggest that previous investigations, which involved injection of ^{3}H-uridine into the animal, rather than into isolated ovarian follicles, failed to detect RNA synthesis in oocytes of antral follicles as the result of precursor pools with too low a specific activity to enable measurement. Additional support for this suggestion comes from experiments carried out with isolated mouse oocytes cultured *in vitro* in the presence of ^{3}H-uridine. Although oocytes freed of cumulus cells by repeated pipetting show only low levels of uridine uptake and incorporation, oocytes surrounded by cumulus cells show increased uptake of uridine and heavy incorporation over the oocyte's germinal vesicle and especially over the nucleolus (Fig. 1 and Table 1). These experiments not only point to the importance of interactions between oocytes and cumulus cells, at least in so far as the entrance of exogenous uridine into the oocyte's intracellular pool is concerned, but also demonstrate clearly that fully-grown oocytes isolated from antral follicles synthesise RNA as long as there is an intact germinal vesicle and nucleolus. In experiments not presented here (Wassarman and Letourneau, unpublished), we find that RNA synthesis ceases concomitant with the onset of meiotic maturation of the oocyte, which includes dissolution of both the germinal vesicle and nucleolus.

Table 1 Uptake and incorporation of ^{3}H-uridine into mouse oocytes

Culture time (h)	C.p.m/Oocyte* +Cumulus	C.p.m/Oocyte* −Cumulus	Grains/GV† +Cumulus	Grains/GV† −Cumulus
1.0	16.0 ± 2.1	10.2 ± 2.2	320 ± 55	110 ± 40
2.0	62.6 ± 14.8	9.8 ± 1.9	1550 ± 450	280 ± 60

* Determination of TCA-soluble ^{3}H-uridine was carried out as previously described[26]. The mean ± s.e.m. for a total of 50 or more oocytes is shown.

† These values represent the mean number of grains per germinal vesicle for 20 oocytes. The values do not include the nucleolar area of the germinal vesicle.

The findings reported here are of particular interest in connection with studies of RNA synthesis during oogenesis in *Xenopus laevis*. For some time it was considered that fully-grown amphibian oocytes are quiescent with respect to RNA synthesis[19–21], attributable, perhaps, to the presence of a specific inhibitor of rRNA synthesis[22,23]. Recent work has, however, shown that these oocytes are not metabolically dormant, but synthesise total RNA at a rate at least as great as smaller oocytes[13]. Two factors which contributed to the impression that fully-grown oocytes do not synthesise RNA are a greatly reduced uptake of exogenous precursors by fully-grown, compared with smaller, oocytes and expansion of the oocyte's endogenous precursor pool which accompanies oocyte growth; both factors can lead to low or undetectable levels of incorporation of radioactive precursors into RNA. The observations reported here suggest that similar factors have influenced the results of previous studies of RNA synthesis during oogenesis in the mammal and have led to an incorrect assessment of the actual situation. Mouse oocytes actively synthesise RNA throughout the period of follicle growth and terminate synthesis only at the time of germinal vesicle and nucleolus dissolution.

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matters arising

Spreading rate in Iceland

WALKER has proposed a new interpretation of Icelandic tectonics in which he recognises the existence of a number of parallel or *en echelon* rift zones[1]. He showed that the aggregate width of the active zones is greatest in southern Iceland and, by postulating that this width is proportional to spreading rate, inferred that the spreading rate in southern Iceland may be three to four times faster than in northern Iceland or the adjacent mid-ocean ridges.

There is one serious objection to this idea. An excess spreading rate in the southern part of Iceland would generate a greater length of lithospheric plate, and either this would have to be taken up in a zone of plate consumption, or else it would result in major compressive deformation of at least one of the plates. There is no evidence that either effect occurs, and I suggest the following explanation of the observations.

The increased widths of young rock outcrops and the greater number of active volcanic centres and thermal fields in the south of Iceland, cited by Walker in support of his hypothesis, are in fact very good evidence of increased magmatism, though they do not necessarily imply an increased spreading rate. The excess material produced can be accommodated by crustal thickening rather than accelerated spreading, thus avoiding the plate-tectonic complications mentioned above. There is abundant geophysical evidence that there is in fact a thickened crust under Iceland as a whole[2], though it is not clear whether the thickening increases towards the south. It seems quite possible that the resultant thicker lithosphere would distribute tectonic stresses over a wider area, giving rise to a pattern of rift zones such as that observed by Walker, and obviating the need to assume that the width of the active zones is proportional to spreading rate.

The same reasoning may be applied to Afar and the Azores, each of which has an excessively thick crust[3,6] and complex rift zones (ref. 4 and D. C. Krause and B. A. McGregor, unpublished) analogous to those described for Iceland. Schilling[5] has proposed that the thicker crusts in all three areas arise from excess magmatism generated by mantle blobs or plumes, and Walker's observations of increased magmatism in southern Iceland represent a partial confirmation of that, and suggest that it is in southern Iceland that the present plume discharge is centred.

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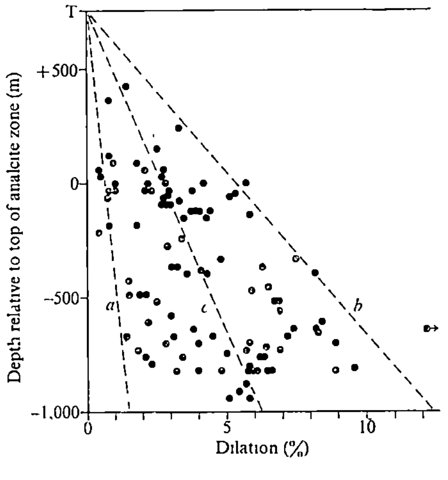
WALKER REPLIES—I fully accept the awkward implications of excess spreading in the southern part of Iceland, as raised by Searle. Some excess spreading could, however, be accommodated by an outward bulging of this part of Iceland, but a determination of its extent must await the delineation of magnetic strip anomalies around the east of the country. Some excess material produced by volcanism could be accommodated by the observed crustal thickening.

An alternative possibility is that basalt is destroyed on a large scale low down in the Icelandic crust, and this is supported by a significant anomaly in the geology of Iceland. This anomaly, the existence of which has apparently not hitherto been pointed out, is the low dyke intensity gradient in the Icelandic dyke swarm. It should be explained that quantitative studies of the Tertiary swarm have been made at many sites in the fjordlands of eastern Iceland[1,2], and everywhere reveal that the dyke intensity increases linearly downwards. On Fig. 1 all the best dyke intensity data, quoted as a percentage dilation, are plotted against depth relative to the top of the analcite zone; this zeolite zone boundary is the most convenient datum level, and is believed to lie 750 m below the original (pre-erosion) top of the Tertiary crust. Much scatter appears on Fig. 1, because at any one level the dyke swarm varies in intensity according to the position of the measuring site relative to local sub-swarms. The intensity gradient varies between 1% dilation per 140 m and 1% per 1,100 m depth, and the median gradient is ~ 1% per 280 m depth.

Fig. 1 Plot of variation in percentage dilation of the dyke swarm against depth in the Tertiary lava pile of eastern Iceland, relative to the top of the analcite zone. Each dot is based on the measurement of dyke thicknesses in a strip of well-exposed country on average 1 km long. *a*, 1% dilation per 1,100-m gradient; *b*, 1% per 140-m gradient; *c*, 1% per 280-m gradient.



A requirement of crustal spreading by the conventional dyke injection model is that the dyke swarm intensity should attain 100% at the base of the crust. Extrapolation of the measured intensity gradient yields 100% dilation at a depth between 14 and 110 km, or 28 km if the median gradient is taken. The crustal thickness is not known for eastern Iceland, but from similar areas elsewhere in Iceland is likely to be ~ 15 km (refs 3, 4). A discrepancy therefore clearly exists: if the median intensity gradient is taken, then > 10 km of crust would seem to be missing.

The magnitude of the discrepancy is critically dependent on the nature of crustal layer 3: whether it consists of metamorphosed basalts[3], or is largely made of intrusive sheets[5]. Layer 3, the lowermost crustal layer, with a P-wave velocity averaging 6.5 km s^{-1}, is found beneath the whole of Iceland and in eastern Iceland occurs on average ~ 3 km below sea level (or 4 km below the original top of the crust). At the top of layer 3 the extrapolated dyke intensity is between 3.6 and 29%, or 14% taking the median gradient. If layer 3 consists largely of an intrusive sheet swarm, then the basaltic lavas in the layer are in effect greatly attenuated by the sheets and the discrepancy is therefore greatly increased: if the median gradient is taken, then several tens of kilometres of crust may be missing.

There is another relationship which seems to support the same general conclusion. It depends on how the total width, D, of dykes injected in a rift zone compares with the total thickness, L, of lava flows produced within the same time period Crustal spreading models assume that $L=D$, which is the ideal situation for steady-state spreading. From the author's experience of volcanism in Iceland it seems more likely, however, that $L > D$, since the typical eruption produces lava totalling tens of metres thick near source, compared with a width of 2 or 3 m for the dyke feeder. An imbalance still remains when dykes in non-eruptive fissures are included.

There is no *a priori* reason why L and D should be equal; L is controlled more by the thermal regime in the mantle immediately below Iceland, whereas D is controlled more by the global stress system, although there is some latitude for a local increase by outbulging and other readjustments within Iceland. If $L > D$, then the crust must inevitably thicken with time. The fact that the Icelandic crust does not thicken in this manner indicates the operation of a counteractive effect destroying crustal material.

Two types of destruction can be envisaged. One type is a general 'ablation' or sapping of material from the base of the crust; the generation of acid magmas by the partial fusion of basalt may be a byproduct of it. The other type is a periodic foundering of big crustal blocks into the upper mantle. The average P-wave velocity in the upper mantle beneath Iceland is only 10% higher than that in crustal layer 3, and the density contrast is likely to be of comparable magnitude. In some circumstances a higher degree of partial melting in the upper mantle might so reduce its density as to cause a density inversion to arise, such that a large scale foundering of layer 3 becomes possible.

There are two prerequisites for the development of a density inversion, namely an appropriate buildup of heat in the upper mantle, and an efficient cooling of the crust so as to preserve its relatively high density. Regarding the latter, the nature of magmatism in Iceland is such as to generate rapidly great volumes of cold rock in the upper part of the crust within and near the active rift zones, and to depress these cold rocks rapidly towards the mantle. The cold rocks consist partly of lava flows which cool rapidly in the air, and partly of dykes intruded above layer 3 or sheets intruded at the top of layer 3, where water cooling by circulating groundwater is extremely effective. Viewed broadly, a high heat loss rate from the Icelandic mantle hot spot is achieved by three main mechanisms besides conductive heat flow: the outpouring of lavas, the emplacement and water cooling of high level minor intrusions, and the rapid downsagging of cold crustal rocks towards, and perhaps their incorporation in, the mantle.

One can speculate that a major foundering episode took place in the southern half of Iceland between 3 and 5 million years ago, and was responsible for a major episode of crustal flexuring (that which formed for example the Lagarfljót flexure zone in eastern Iceland[2]), and for the birth of some of the multiplicity of rift zones in the resulting thinned crust of southern Iceland[6], besides perhaps facilitating outbulging to accommodate the increased spreading there.

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Aromatic structures in coal

THE demonstration by Hayatsu *et al.*[1] that dichromate oxidation of coal allows isolation of clearly identifiable hetero-aromatic entities is important because very little is so far known about such structures in coal. But it does not by any means invalidate our inference[2] that the skeletal structure of coal is predominantly non-aromatic, and is, in fact, entirely consistent with that view.

Bearing in mind how susceptible coal is to oxidation by air at temperatures as low as 70 °C, autoclaving it with aqueous dichromate at 250 °C for 36 h is hardly a mild treatment, and raises the distinct possibility of pyrolytic cleavages and major structural rearrangements. We note, in this connection, that there is a large body of evidence that coals other than anthracites begin to suffer substantial thermal decomposition well below 200 °C.

Assuming that the carboxylic acids identified by Hayatsu *et al.* did not form pyrolytically set up polynuclear 'artefacts', considerable interest attaches to the carbon balance which quantifies their findings. From the nature and yield of their oxidation products we calculate that at most 45% of the original carbon in the coal was recovered in the carboxylic acids. The remainder was lost to CO_2 and since dichromate does not, as Hayatsu *et al.* correctly point out, cause significant ring degradation, this must have come from non-aromatic (Sp^3 carbon) structures. Moreover, since the carboxylic moieties must, for the same reason, also have formed from aliphatic and/or alicyclic linkages, at least 25% of the carbon in the carboxylic acids must likewise be assigned to the Sp^3 valence state. Overall, then, the results obtained by Hayatsu *et al.* indicate that some 65% or so of the total carbon in coal exists in the Sp^3 state—which coincides almost exactly with our estimates from hypohalite oxidation of coal.

It appears that all coal oxidation studies, regardless of what oxidants and reaction conditions are used[3–5], lead to substantially similar products and strikingly similar $Sp^3:Sp^2$ carbon ratios (see Table 1). Were one to assume that coal is mostly comprised of polycyclic aromatic structures, one would therefore be forced to conclude that weak oxidants, such as sodium hypochlorite ($E_B^0=0.89$) or dichromate ($E_B^0=1.2$) can oxidise polycondensed aromatics as readily as alkaline potassium permanganate ($E_B^0=1.69$) or concentrated nitric acid—a conclusion that is inconsistent with what Hayatsu *et al.* correctly designated for dichromate oxidation. If nothing else, the sensitivity of coal to oxidation and the fact that the strength of the oxidant is not a prime determinant of the vigour of the reactions should be enough to suggest a predominantly non-aromatic structure. Relatively asymmetrical, branched 'diamondoid' skeletal structures, which are much more easily oxidisable than polycondensed aromatics with conserved symmetry, would certainly be more consistent with experimental observations.

In these circumstances, we conclude that discovery of C_{10}-, C_{14}- and C_{18}-polycyclics among the oxidation products of coal, if confirmed by less drastic means than those used by Hayatsu *et al.*, only requires a review of earlier reports that oxidation never delivers anything beyond single-ring compounds (and CO_2).

Table 1 Distribution of carbon in oxidation products (as percentage of coal carbon, Pittsburgh seam)

Products	Alkaline $KMnO_4$ (various conditions)	$NaOH+H_2O+O_2$ Press 7–25 atm 100–250 °C	$Na_2Cr_2O_7+H_2O$ Autoclave 250 °C	$NaOCl+H_2O$ 60–65 °C Open flask
Carbonate carbon	45.0	50.0	65.0	63.0
Oxalate carbon	15.0	13.0		
Aromatic carbon	31.0	36.0	35.0	37.0

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HAYATSU ET AL. REPLY—First, with respect to the ease of oxidation of coal by air at 70 °C we need only quote from ref. 1: "For example, at 180 °C and oxygen concentrations <20%, conversion to humic acids is negligible, and significant reaction rates at this temperature are only observed when [O] >50%".

Detailed pyrolysis studies of the bituminous coal we used for our experiments showed no evidence of thermal decomposition until the coal was heated well above 250 °C.

Second, the estimate of the degree of aromatic structure from our data is much too low for the following reasons: (a) Because of the large concentrations of salts resulting from oxidation with $Na_2Cr_2O_7$, yields were not quantitative and were estimated to be ~75%. As we mentioned in our paper the amphoteric acids were not isolated from this oxidation but were identified in the product from a photochemical oxidation with much less interfering residues; (b) a significant fraction of the aromatic compounds appeared in a neutral fraction, and (c) as Friedman has shown, aromatic rings containing phenolic groups would have been destroyed by $Na_2Cr_2O_7$.

By a method based on the stoichiometry of fluorination reactions (J. Huston, R. G. Scott, and M. H. Studier, unpublished) we have calculated the aromaticity to be 69%.

Third, we agree that the strength of an oxidising agent is less important than its specificity in studying coal structure. Friedman[2] has demonstrated the selectivity of aqueous sodium dichromate in an autoclave at 250 °C without pyrolytic cleavage or structural rearrangements occurring. Several authors[3-5] have shown the nonspecificity of aqueous NaOCl, the reactions of which are the basis of Chakrabartty's and Berkowitz's postulate that coal is primarily non-aromatic.

We feel it more likely that coal consists of aromatic units bridged largely by aliphatic and hydro-aromatic groups than that it has a 'diamondoid' structure.

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Trapped helium and argon and formation of the atmosphere

FISHER[1] reports ^{4}He/^{40}Ar ratios from analyses of the glassy margins of several deep sea basalts which are higher than can be generated by the *in situ* decay of K, U and Th in the mantle if one uses conventional estimates for the K/U and Th/U ratios in the mantle. He then concludes that "the data rule out all models which ascribe the terrestrial atmosphere to any type of degassing of a mantle which is chondritic or crustal with respect to the K/U ratio". While this sweeping conclusion may ultimately prove correct, Fisher's argument is suspect since it involves an assumption which is probably false. The assumption is explicitly stated: "The rare gases found in deep sea basaltic glasses are clearly those trapped within the rock on its eruption, due to the high hydrostatic pressures and rapid chilling, and as such represent mantle-ambient gases".

Oceanic basalt magmas are commonly taken to be fractionated (by partial melting) samples of the mantle. Ozima and Alexander (unpublished) have argued that the partial melting process produces large enrichments of light rare gases relative to the heavy rare gases in the melt phase. For example, Gramlich and Naughton[2] have shown that Iherzolite nodules, which they conclude represent unfused residue from the partial melting process, contain ^{4}He/^{40}Ar ratios lower than reasonable values. If the residue is depleted in ^{4}He relative to ^{40}Ar, simple mass balance requires that the melt phase be enriched in ^{4}He relative to ^{40}Ar. While data from the glassy rims do probably represent lower limits on the ^{4}He/^{40}Ar ratios in the magma, the magma values are only bad upper limits on the mantle–ambient values. The magma ^{4}He/^{40}Ar ratios do not, therefore, rule out the degassing models mentioned above.

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FISHER REPLIES—The suggestion of Alexander[1] (based on unpublished work of Ozima and Alexander) that the ^{4}He/^{40}Ar ratio may be quantitatively enriched in the mantle–magma segregation process is certainly interesting, though unproven. Alexander's suggestion that Gramlich and Naughton[2] concluded that their samples represent residue from the partial melting process with He depleted relative to Ar, and therefore that the melt phase must be enriched in He relative to Ar, ignores Gramlich and Naughton's actual interpretation of their data: that their gas ratios were probably "altered during a high temperature episode such as that existing during transport in the erupting lava"[2].

We know that such a high temperature episode existed (the eruption itself), and that this would segregate He from Ar; therefore we cannot use these data as evidence for another and previous segregation process, as Alexander would like. The simplest interpretation of my data remains that the measured ratios represent those in the source material except as modified during eruption; that is, they are lower limits to the mantle–ambient ratios. Experimental investigations that might prove the reality of the Ozima–Alexander model will be welcomed.

Alexander (personal communication) has pointed out an error in Fig. 1 of my paper[3]: the values for all curves below 10^7 yr should be levelling off, not decreasing. This does not affect in any way the arguments presented either there or here.

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Ambiguous cognitive contours

BRADLEY and Dumais[1] have presented figures that induce ambiguous subjective contours, which tend to alternate between two (or more) perceived forms. Bradley and Dumais suggested that because these figures are not "stimulus-bound", they cannot be accounted for by a physiological model, but arise when alternative perceptual hypotheses are tested[2]. Although this account is plausible, a more physiological explanation may also exist. The alternation of perceived forms resembles an effect described by Campbell *et al.*[3,4]: if two gratings that have sinusoidal luminance profiles are projected so that their axes are at an angle to one another, the gratings tend to be seen in alternation, one replacing the other at intervals of several seconds. Campbell *et al.* suggest that this occurs because while one grating is perceived, the channel that is most sensitive to that grating becomes adapted. When adapted, its sensitivity decreases[5], allowing the other grating to predominate.

When observed steadily, Bradley and Dumais' patterns undergo a similar alternation. This is seen most easily in their star pattern, in which the two triangles predominate alternately. It is difficult or impossible to maintain the perception of one triangle, although if they had a cognitive origin, one would expect to be able to do so. Furthermore, an adaptation hypothesis can better account for the fact that the figure that is predominant at a given moment appears brighter. If adaptation takes place, it is reasonable that the less strongly adapted figure should be brighter. It thus seems premature to conclude that this phenomenon cannot eventually be accounted for by a physiological model.

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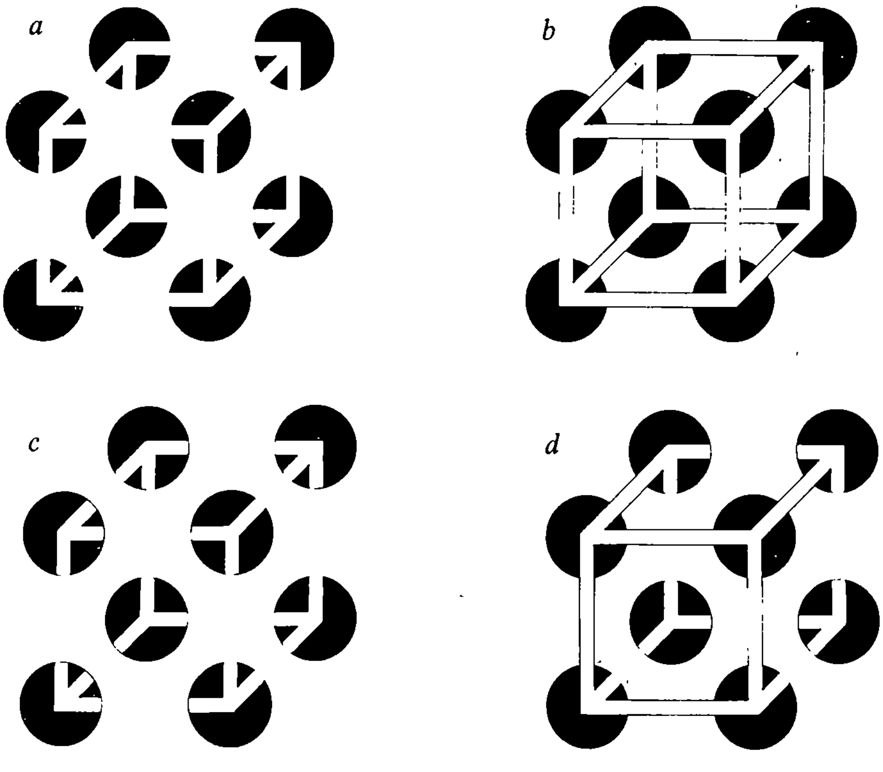
BRADLEY, DUMAIS AND HEYWOOD M. PETRY REPLY.—If Cavonius is correct in attributing the alternating organisations of our ambiguous configurations to the adaptation of "the channel that is most sensitive to that grating" (ref. 1), then such configurations should alternate independently of the viewer's knowledge of the possible organisations. It is not difficult, however, to construct figures which are highly resistant to alternations until the viewer is sensitised to the existence of alternative organisations. An example of such a figure, called the subjective Necker cube[2], is presented in Fig. 1*a*. If observers are permitted to look at this figure for an extended period, most will eventually report seeing a cube overlying eight black disks and a white background (as in the unambiguous version of Fig. 1*b*). The whiter-than-white bars of the cube seen extending between the disks are, of course, illusory and are bounded by subjective contours.

Having achieved this cube-in-front organisation, most observers will maintain it indefinitely. If shown the unambiguous cube-in-back organisation of Fig. 1*c*, however, they then report being able to see the cube in Fig. 1*a* in that position as well, with subjective contours now bounding the interior edges of the 'holes' of the occluding surface rather than extending between the disks. Of course, once aware of these two organisations, the observer will then experience spontaneous alternation between the cube-in-front and cube-in-back. Although this might be accounted for by an adaptation hypothesis, such an hypothesis could not account for the initial absence of alternation. Furthermore, the observer can be 'cued' to a third possible organisation; that depicted in Fig. 1*d*. In this case, the observer will see both straight and curved subjective contours in Fig. 1*a*, because the 'straddled' organisation cued by Fig. 1*d* puts part of the cube in front and part in back. It seems reasonable to suppose that perceptual phenomena which are strongly affected by set or expectation probably have a cognitive origin.

Fig. 1 Unambiguous versions of three possible organisations of the subjective Necker cube. *a*, Original ambiguous version; *b*, cube-in-front; *c*, cube-in-back; *d*, straddled.



This view does not preclude the possibility of a physiological explanation of subjective contours. It does, however, require that any such explanation be able to incorporate the effects of cognitive variables (set, expectation, attention and so on) on the manner in which subjective contours are perceived in ambiguous displays.

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Insectivorous grouse

IT is, perhaps, unfortunate that we did not publish the results of incubating hens and other adult grouse separately in our paper on insects as food of adult grouse[1]. In 1974, droppings from four incubating grouse on wet moors (at Moor House, Cumbria) were examined and contained similar proportions of insect remains (mainly tipulids) as did non-incubating birds. Similarly, females with young of different ages all showed tipulid remains in their droppings. Thus Moss and Watson[2] can rest assured that incubating and brooding female red grouse do, at least on occasion, feed on tipulids in addition to vegetation.

Moss and Watson go on to state "there is no evidence that tipulids are ever in short supply on wet moors". We have information to the contrary[3–5]. In addition, both *Molophilus ater* and *Tipula subnodicornis*, the main species involved, fluctuate markedly in numbers and larval densities between 2,500 m^{-2} and 50 m^{-2} in the former and 250 m^{-2} and 5 m^{-2} in the latter have been documented by us on several occasions.

The relationship between grouse numbers and tipulid densities on wet moors is unknown. Even the cautious research workers might consider investigating the possibility of such a relationship, particularly since it has been shown that N and P, which are in much higher concentrations in tipulids than in *Calluna*, are important to grouse[6].

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reviews

IBP–nitrogen fixation

Christina Kennedy

Nitrogen Fixation by Free-Living Micro-organisms. (International Biological Programme 6.) Edited by W. D. P. Stewart. Pp. xxi+471. £13.50. *Symbiotic Nitrogen Fixation in Plants.* (International Biological Programme 7.) Edited by P. S. Nutman. Pp.xxviii+584. £22.00. (Cambridge University: Cambridge, London and New York, 1976.)

An outstanding feature of the IBP synthesis meeting on nitrogen fixation held in Edinburgh in September 1973 was its international character. That very strength created a potential weakness as far as publication of the proceedings was concerned, and I remember sometimes labouring over abstracts at that meeting wondering just what it was that I had come to learn about. Messrs Stewart and Nutman, the editors, are therefore to be congratulated on the high standard of English and easy readability of both these volumes which are largely based on that IBP meeting. Both volumes contain a mixture of review articles, original research papers and a few compiled results of surveys. The reader must beware, however, of chapter titles that belie their actual contents.

The first two sections of volume 6 give roughly equal time to the two major groups of free-living nitrogen fixers, certain bacteria and blue-green algae. The reports of field research in Brazil (Dobereiner and Day, ch. 3) and in the UK (the longstanding Broadbalk experiments at Rothamsted; Day *et al.*, ch. 5) are elegant examples of how to assess the ecological importance of bacterial nitrogen fixation, but this section would benefit from an overview of the relative importance of the various groups of free-living nitrogen-fixing bacteria. There is also very little about the regulation or physiology of fixation in bacteria other than azotobacters.

If we are left wondering about the overall contribution of non-symbiotic bacteria to the world's agricultural nitrogen balance, there can be little doubt about the usefulness of blue-green algae in certain agricultural situations. These are extensively covered in volume 6 and complemented by the excellent chapters in volume 7 on the symbiotic associations of blue-green algae with plants. It is a pity that all this work is not included in the same volume in spite of the different modes of fixation.

Volume 6 continues with a section on the use of acetylene reduction as a measure of nitrogen fixation which, although useful, is not the last word, as equally valuable descriptions of this technique are described throughout research articles in both volumes. The final biochemistry section contains some well-written, if outdated, reviews and research reports on nitrogenase (this faster-moving branch of nitrogen fixation has been often reviewed recently). Still timely is ch. 26 on reductants for nitrogenase. Hardy *et al.* remind us (ch. 24) that there is another world of relevant research on the chemistry of dinitrogen complexes.

Two chapters in the biochemistry section of volume 6 describe the serological identification of rhizobia and leghaemoglobin biosynthesis and therefore could usefully have been swapped for the opening three chapters on the genetics of nitrogen fixation in free-living bacteria found in volume 7 (also a little late to be useful since most of the data has appeared elsewhere by now). We find that *Rhizobium* genetics is in a primitive state by comparison.

The rest of volume 7 describes nitrogen fixation in symbiotic systems, both legumes and non-legumes. It includes sections on legume inoculation (reviews and research reports), legume field experiments (mostly research reports on plant yields versus inoculation or fertilisation), and reactions of legumes to environmental stress (including legume physiology) The final section includes non-leguminous root symbioses and the aforementioned blue-green algal–plant associations. The negative results of chapter 39 should kill the oft-sought, oft-cited nitrogen fixing role of nodules found on certain tropical leaves.

On the whole these volumes contain an unprecedented collection of information and references. Access could have been improved by better indexing, especially since some chapter titles are misleading. Since the books will probably be bought as the set, cross-indexing would have been more useful than the multi-lingual tables of contents. They are burdened with the usual disadvantages of large-scale publishing enterprises in being too expensive and too late. The meeting was in 1973 and as a consequence neither volume refers to areas of more recent importance: the role of glutamine synthetase as a regulator of nitrogen fixation, fixation by *Rhizobium ex planta* (or even in callus culture) or nitrogen fixation by *Spirillum lipoferum* in maize plants.

Finally, volume 7 closes with excerpts from the meeting's last session which was a colourful, sometimes heated, sometimes amusing discussion of possibilities for extending nitrogen fixation to other plants. Unfortunately that atmosphere dosen't come through. The written version of Ray Valentine's comments that "exploratory work . . . is not necessarily expensive" just doesn't make it since what he really said was "doing this stuff will only cost a few bucks." ☐

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New power horizons

G. R. Bainbridge

Energy: The Solar Hydrogen Alternative. By J. O'M Bockris. Pp. xviii+365. (Architectural Press: London, February 1976.) £13.50.

Practical scientists and engineers, energy planners and policy makers, to whom the author says his book is directed, will have no difficulty while reading it in coming to the conclusion that economic, large scale solar energy and hydrogen fuel systems are still many decades away. The uncertainties about continuing supplies of fossil fuels, including the problems of extracting, transporting and applying them economically will lead to increasing research and development work into several alternatives: in particular, solar and hydrogen.

The students of energy conversion, the environmentalists and people concerned in an effort to prepare for the coming era of difficulty, in which a change in energy source has possibly to be made from the diminishing fossil fuels, might reasonably be excused if they remain confused after trying to read the multi-faceted presentation from cover to cover. The book contains a bit of everything in energy; atomic, wind, geothermal, hydro and tidal illustrations are all martialled sequentially, as well as the main subjects of the book, to help to answer a whole host of questions about the rate at which abundant 'clean' energy might substitute for diminishing resources of coal, oil and natural gas. The oil and gas, warns the author, may be scarce and expensive within a few decades and the demise of coal may be only a few decades later. Nuclear fission and fusion systems will have their problems and may not be sufficient if the time before there is a real need for them is shorter than some people expect.

The propositions concerning doom in the 21st century due to increasing CO_2 and aerosols in the atmosphere, from the burning of fossil fuels, affecting world climate are brought out, as are the prospects of NO, CO and SO_2 causing substantial health hazards. The idea of the delayed and therefore necessarily nimble changes in technology and engineering being achieved to avoid catastrophe by providing abundant new forms of energy, are not expected to be realised. Practical population control and the highly efficient recycling of materials to avoid shortages are not viewed hopefully. Experience indicates that modifications in the world energy mix and social behaviour patterns are slow and will continue to be so.

How soon in the future will ideas of the solar–hydrogen economy be strongly tested? Will the money, the manpower and materials necessary be made available by Governments for building large remote land-based, or offshore, atomic and solar power stations? If they can be, the scale of expenditure is bound to be limited. The reader may have doubts about this happening significantly in view of the recent slowing down of fission and fusion power research and development work. The stages outlined by Bockris seem then to be far too ambitious and to deviate too far from a main line of attack on the main solar electric and hydrogen production problems.

On these matters the reader is best left to form a personal judgement, to assist which some of the multitude of references so excellently given could be consulted, at a rate averaging about 1.6 per page of text. □

Solar connection

Possible Relationships Between Solar Activity and Meteorological Phenomena. Edited by W. R. Bandeen and S. P. Maran. Pp. ix+263. (NASA: Washington, DC, 1975; sold by US Government Printing Office.) $4.00.

THIS book marks the official publication of the proceedings of the NASA Symposium on possible relationships between solar activity and meterology held at the Goddard Space Flight Center in November 1973. I share the disappointment of NASA's Scientific and Technical Information Office concerning the delay in publication, as this was clearly an important conference in defining the starting ground for much of the current work on solar–weather links. Publication in 1974 would have been of great value to those investigating these links who were unable to attend the conference. Two years later, its value is much diminished.

Since the proceedings have been available in preprint form since June 1974, at no charge, and the book costs $4, it might seem that the only recommendation for this edition is that it is more compact than the preprint. The papers are often brief to the point of superficiality and in many cases very subjective, whereas the field of study is very rapidly becoming a much tighter and more objective discipline than it was up until recently. S. I. Rasool aptly summarises the conference: "What we do not want is a symposium where there are 5-minute presentations and where one cannot ask a question without a microphone". Nor, in 1976, do we want a book which reports uncritically the proceedings of such a symposium on this important topic.

John Gribbin

Authoritative text on quantum electronics

Quantum Electronics: A Treatise. Edited by Herbert Rabin and C. L. Tang. Vol. 1: Non-Linear Optics, Part A Pp xi+472+9. $35; £17.50. Vol. 1 : Non-linear Optics Part B. Pp. ix+473–753. $22 50; £11.25. Academic: New York and London, December 1975.)

THE nine chapters of these long-awaited volumes cover substantial ground, enough, as Bloembergen remarks in a general introduction, to catch the spirit of non-linear optics without exhausting its content. Common chapter organisation and notation, frequent cross-referencing, a universal index, all in attractive format, make for good consultation.

Part I (Non-linear Optical Susceptibilities) is mostly taken up with an encyclopaedic survey by Christos Flytzanis of the Theory of Non-linear Susceptibilities. The author presents their phenomenological properties and evaluations in massive mathematical detail. Generalisations then give way to minute discussion of individual models relating to rarefied and condensed media. Some 500 references complete the review. The Measurement of Non-linear Optical Susceptibilities by Stewart K Kurtz complements the previous theory. After a prefatory review of Gaussian beam optics and light propagation in anistropic media, there follow practical descriptions of the absolute methods of phase matching, parametric fluorescence and Raman scattering; and the relative methods of Maker fringes, and wedges.

Introducing Part II (Non-linear Optical Processes), Two-Photon Absorption Spectroscopy by H Mahr is a lucid account of the underlying physics of symmetry, polarisation and resonance applied to molecular and electronic transitions. Experimental results in solids, liquids and gases are surveyed. I. L Fabelinskii reviews experiments and practical theory relating to the Stimulated Mandelstam–Brillouin Process Studies of electrostrictive coupling, stimulated temperature process, non-linear absorption are related to a variety of stationary and transient phenomena. A short essay by C. L. Tang on Spontaneous and Stimulated Parametric Processes explains the basic theory and experimentation of parametric fluorescence, and discusses its applications. Concluding Part II, Chen-Show Wang writing on The Stimulated Raman Process presents theoretical studies of the Raman coupling of pump and scattered light with mechanical mode excitation, both in forward and in backward transient stimulated scattering. Related effects and experimentations are briefly discussed.

Part III (Applications) opens with a substantial review by S. A. Akhmanov, A. I. Kovrygin and A. P. Sukhorukov of Optical Harmonic Generation and Optical Frequency Multipliers. This chapter concentrates on the theory and design of optical frequency multipliers, and divides into steady-state and transient generation, the latter including ultrashort laser pulses. In Optical Parametric Oscillators (Robert L Byer), theory and experimentation are elegantly blended into a complete exposition of operation characteristics, devices and materials In the final chapter by John Warner, the technique of Difference Frequency Generation and Up-conversion are briefly explained, the latter mainly for signal and image detection.

These volumes should form an authoritative text for some years.

P. G. Harper

Mathematical explanation

Introduction to Mathematical Biology. By S. I. Rubinow. Pp. xiii+386. (Wiley-Interscience: New York and London, December 1975.) $27.00; £13.55.

THIS book is based on a course designed to develop in graduate biology students at Cornell University an awareness of and familiarity with some mathematical techniques and methods applied to biology. Apart from brief mention of the application of the least squares method to linear regression and the extraction of the parameters of multi-exponential processes by the 'peeling' method, statistical and numerical analytical techniques for processing experimental data are not featured. The author clearly believes, however, that mathematical biology should be firmly based on experimental observation. Consequently, no attempt is made to describe methods involving analogue or digital computers for handling speculative mathematical models of biological systems.

The book deals with the mathematical explanation of five selected biological phenomena in some depth: cell growth, enzyme kinetics, tracers in physiological systems, biological fluid dynamics and diffusion in biology. The style is economical so that, for example, steady-state and pre-steady-state kinetics, cooperativity and allostery and the effects of inhibitors and modifiers are treated in 53 pages in the chapter on enzyme kinetics.

Where possible, the author applies fundamental concepts to a variety of biological problems. For example, an explanation of Fick's laws of diffusion leads to consideration of topics as diverse as the relationship between diffusion constant and molecular weight, pheromone interaction between animals, membrane transport, diffusion through a slab, axonic ion flow, ultracentrifugation and diffusion across capillary walls. Three other features of the book deserve mention. When a new term is introduced and defined, it is printed in bold-faced type which will facilitate revision by students. Each chapter ends with a number of problems of graded mathematical difficulty. The solutions with full explanations are given towards the end of the book.

Finally, although the author states that this is not a textbook in mathematics, there are two useful mathematical appendices for readers who have forgotten basic techniques such as the handling of matrices and determinants. Unfortunately, students of the biological sciences who could and should read this book are likely to find the price a deterrent. It deserves a place in science libraries and hopefully will be well thumbed with use by students and lecturers.

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PMO theory

The PMO Theory of Organic Chemistry. By Michael J. S. Dewar and Ralph C. Dougherty Pp. xiii+576 (Plenum: New York, 1975.) $14.50.

IN the preface, the authors disclaim any intention of rewriting the whole of organic chemistry in terms of perturbational molecular orbital (PMO) theory. The text illustrates principles of such widespread application that it seems that only time has prevented them from so doing. The first three chapters introduce the theory of the method in a most readable manner. The principles are well presented, and the material could stand up in its own right as a separate text. The bulk of the text comprises three chapters with far-reaching titles —Chemical Equilibria (ch.4), Chemical Reactivity (ch.5) and Light Absorption and Photochemistry (ch.6). The final chapter is concerned with transient ions, and seems too specialised for the general audience at whom the work as a whole is aimed. Inevitably some of the material will provoke controversy, and there is a rather cavalier treatment of alternative approaches to some of the problems For example, the summary dismissal of the Hoffmann–Woodward approach to Pericyclic Reactions, and the absence of any reference to R. W Taft in the section dealing with substituent effects would be remarkable if it were not so predictable. The book succeeds admirably in its stated aim of demonstrating the power of the PMO method, and does so in such a readable manner that it will be appreciated by a wide audience.

L. Phillips

Infrared astronomy

Infrared: The New Astronomy. By David A. Allen. Pp. 228. (Keith Reid: Sheldon, Devon, November 1975.) £6.50.

THIS is an unusual book, a semi-popular one written completely from the viewpoint of the observational astronomer. It charts the rise of a new field, infrared astronomy, with which the author has been actively involved for a decade. After introductory chapters outlining a few background ideas, the techniques of observation (formidable if the only dewar you have ever handled is a thermos flask), the main observing sites, and the early history, the book discusses the different types of source seen in the infrared: solar system objects, stars and protostars, and galaxies and quasars. All are discussed on the basis of whether they are detectable in the infrared, and more particularly whether they are unusually strong emitters there. This seems to be the weakness of the observer's viewpoint, especially when he is specialising in a fairly narrow range of wavelengths (most of the book concerns the range 1–20 μm). No theoretical framework is provided which explains why different types of star or galaxy are of interest. So this is a book which requires a rather extensive knowledge of astronomy to appreciate its qualities.

The chapters on the different types of star which radiate in the infrared, either because they are cool or more often because they are partially shrouded in dust, are outstanding, and constitute a valuable review of, and introduction to, the field.

That on galaxies and quasars, on the other hand, opens weakly: "A galaxy is some breccia constructed of all the types of object we have encountered in the last four chapters". No real effort is made to distinguish between different types of galaxy. Under "Compact Galaxies" we find I Zw 1727+50, which is also a BL Lac-type object: the "Markarian galaxies" mentioned are also, in fact, Seyferts And the chapter ends with a decidedly casual (and even for June 1974, inaccurate) account of the microwave background.

If Dr Allen were writing the book today, I think he might devote a larger fraction to wavelengths between 30 μm and 1 mm, where many of his sources emit most of their energy. He might also devote more space to the clouds of dust and molecules out of which form the young stars on which he is so expert.

This is a book for the professional astronomer (for whom an excellent feature will be the comprehensive list of references) or the knowledgeable amateur. How the style appeals to you will depend on your taste for extravagant metaphor. For example, synchrotron radiation is explained as "those piercing shrieks emitted by high energy electrons when they embark on their helter-skelter paths in magnetic fields"

M. Rowan-Robinson

Lemurs

Lemur Biology. Edited by Ian Tattersall and Robert W. Sussman. Pp. xiii +365. (Plenum: New York and London, 1975.) $27.54.

THIS is a progress report of recent studies. To emphasise the breadth of modern interest in lemurs, studies from several disciplines have been drawn together. There are five sections: an introduction to lemur biology (the history of the study of lemurs; the Madagascan environment); systematics and evolution (comparative studies of chromosomes and teeth), morphology and physiology (studies of cranial anatomy, postcranial skeleton and body temperature); behaviour and ecology (studies of several little-known lemurs inluding *Indri, Phaner, Hapalemur* and *Propithecus*); and an epilogue on conservation and extinction. The editors emphasise that "the interpretations in each of these fields are dependent on knowledge of each of the others". It therefore seems reasonable to expect them to promote the cross-fertilisation of disciplines by ensuring that individual contributions are understandable to the non-specialist. Although most papers are admirable in substance they leave the non-specialist confused by repeatedly using specialised jargon (for example, terms from cytogenetics, dental and postcranial morphology, behaviour) which is either inadequately defined or not defined at all. Most readers, being less familiar than the authors with Madagascar, may also find difficulty in following the text because of the lack of maps showing topographical reference points and the distribution of species. The usefulness of this style of book is limited unless it is something more than a collection of papers of the type found in specialist journals.

In spite of those criticisms I enjoyed *Lemur Biology*. Its breadth of coverage is refreshing and instructive There has clearly been a delay in publication and the preface sensibly records the most recent developments.

John M. Deag

Down to Earth

Man's Relation to the Universe. By Bernard Lovell. Pp. vi+118. (Freeman: San Francisco and Reading, November 1975.) $5.95.

DURING 1973, Professor Bernard Lovell delivered a series of lectures on astronomy and space research to students at Buffalo University. The content of these lectures is available in an informative little book which carries the somewhat misleading title of *Man's Relation to the Universe*

Indeed the first chapter is very much down to Earth, with an unusual and entirely welcome discussion about the financial cost of space-age astronomy. We are told such horrors as the (certainly astronomical) cost—$400 million—of the Soviet 236-inch telescope, although to put it all in perspective, the UK government turns out to spend only 0.02% of the gross national product on all forms of astronomical research.

Stepping into near space Professor Lovell skillfully summarises the results gleaned by the recent space probes to Mercury, Venus, Mars and Jupiter. The explosion of information about these planets is bewildering, but the author delivers a well-balanced and cautious appraisal of contentious issues, such as the 'water on Mars' controversy, but without losing a sense of excitement and enthusiasm.

Moving farther afield, stellar and galactic evolution are described, and modern discoveries such as quasars, pulsars and (possibly) black holes all get a mention The discussion is somewhat formal, but never dull or over extended. In spite of the title, there is very little hard philosophy, except on cosmological issues such as the big-bang creation event.

I very much enjoyed reading this book. Laymen of a scientific inclination may find rather too much technical discussion to cope with the content without preparation. Scientists and students will find it a straightforward and engaging introduction to the techniques and results of modern astronomical research.

Paul Davies

Aquatic fungi

Recent Advances in Aquatic Mycology. Edited by E. B. Gareth Jones. Pp. 749. (Elek Science: London, December 1975.) £21.

THE idea for this volume grew from the success of seven sessions devoted to aquatic fungi at the First International Mycological Congress held in Exeter in 1971, and some of the chapters are papers presented at the Congress. It is disappointing that it has taken four years for the book to appear, so that some of the advances are by now hardly recent.

About half of the book, Section I, is devoted to marine fungi; the rest, Section II, to freshwater forms. The marine fungi are considered under two arbitrary divisions—A, Ascomycetes, Fungi Imperfecti and Basidiomycetes; and B, Lower Fungi. Section IA has chapters on lignicolous fungi, mangrove fungi, oceanic yeasts, fungal degradation of oil, physiology, and cytochemistry of spores. I particularly enjoyed the account by J. W. Fell of oceanic yeasts, some of basidiomycetous affinity, which brought together, in a coherent way, material from widely scattered literature of a group which will be unfamiliar to many. Section IB, mostly covering zoosporic forms, includes a general account of marine 'phycomycetes', an essay by F. K. Sparrow on the present status of classification in biflagellate fungi (not exclusively marine), and chapters on fungal diseases of marine animals, physiology of marine 'phycomycetes', fine structure, and ecology. The freshwater Section is also divided into two arbitrary divisions. The section on Ascomycetes, Fungi Imperfecti and Basidiomycetes has chapters on morphology and biology, fungi from cooling towers, sewage, an interesting account of the role of Hyphomycetes as intermediaries of energy flow in streams, and on the interactions between river fungi and DDT. The 'lower fungus' section includes chapters on the morphology of Chytridiomycetes in relation to substrates, fungi on algae, ecology, physiology, aspects of fish disease, ultrastructure, and a valuable review of the Trichomycetes by R. W. Lichtward. Aquatic Actinomycetes, although accepted as prokaryotic, are included in a useful chapter by L. G. Willoughby.

The general impression of the book, apart from the uneven quality inevitable in symposium-type publications, is that it will provide useful reference material for those working on aquatic fungi. The photographic illustrations leave much to be desired, especially with respect to size, layout and quality of reproduction. The use of unglazed paper and the faint printing has rendered many of the electron micrographs virtually useless, as is shown in the otherwise excellent article by Heath on ultrastructure of freshwater Phycomycetes. Many of the plates have obviously been made up by their authors to fill a whole page, but have been reduced by the publishers to take up only a small fraction Much of the space 'saved' by this unfortunate economy has been wasted by the use of large print and author citations in voluminous tables. Moreover, there are numerous misprints The book will prove far too expensive for many individuals to buy.

John Webster

obituary

The news of **Michael Polanyi's** demise caused deep sadness in the hearts of his many friends and admirers. There were, and are, few people in our century whose interests were as extended as were his, whose friends were as devoted to them as were those of "Misi".

Polanyi was born in Budapest, Hungary, on March 11, 1891. He took his degree as an MD in Budapest at the age of 22, but wrote his first paper (on the hydrocephalic liquid) long before that, at the age of 19. His interest then centred on biology, principally human biology, but his articles soon became sprinkled with references to, and conclusions drawn from, physical chemistry, and, in particular, the second law of thermodynamics. The first such paper was written in collaboration with a life-long friend, J. Baron, but the other papers written during the first world war, when he served in the army, were by him alone. They were concerned not only with applications of the second law, but also with the foundations of the third law, then called Nernst's Theorem. His last article during the war period, however, was on a subject which remained long close to his interest: the theory of adsorption.

Soon after the end of the war, Polanyi moved to Germany—for a year to Karlsruhe, where he met his wife, then to Berlin where he worked first at the Kaiser Wilhelm Institut für Faserstoffchemie (chemistry of fibres), and then at Haber's Institute, the institute for physical and electrochemistry. He moved to Manchester, England, in 1933, when Hitler came to power, a reason very similar to that which had originally prompted him to leave Hungary.

The breadth of Polanyi's interests manifested itself in his days as a scientist by the wealth of subjects to which he made important contributions. Several of them continued to attract his interest virtually all through his scientific career. His first articles on the subject mentioned before, the adsorption of gases to solid surfaces, were written in 1914 and 1916, the last one referring to this subject in 1946. His chief interest was, however, concentrated on the rate of chemical reactions. He contributed decisively both to the theory and to the development of experimental methods to investigate them. His first paper on the subject was written in 1920, a theoretical paper which was later fully justified by quantum mechanical considerations in 1925 and his concluding paper on this topic dates from 1949. In addition to these, he contributed significantly to our understanding of the strength of materials and the role of the crystalline glide-planes in this connection, to the X-ray analysis of fibres and crystals, also of poly-crystalline materials and, finally, to simple chemistry (and perhaps to other subjects which this writer fails to recall).

Two thirds of Polanyi's scientific articles written after 1920 (he published 201 such articles) were published jointly with collaborators, some of whom have acquired world fame. He not only stimulated interest in his collaborators, he also established close personal relations with most of them and they maintained their devotion to him and his ideas throughout his life. This applies also to the present writer.

Eugene P. Wigner

Were it not that he was one of the great polymaths of this century, it would be surprising, as it was apparently regrettable to some of his science colleagues, that Michael Polanyi, after coming to the University of Manchester in 1933 as Professor of Physical Chemistry with a great international reputation in science, should in 1948 have been appointed as Professor of Social Studies. Yet it is intriguing to speculate as to why he made the decision, with the general approval of his University and the warm welcome of the economists there, to shift the balance of his intellectual interests. Running through his writings in economics—USSR Economics in 1935; Patent Reform 1944; Full Employment and Free Trade in 1945; Profits and Polycentricity in 1946; and The Span of Central Direction 1948—there seems to be one leading strand: how best to reconcile the safeguarding of individual liberty with the controls upon the individual inseparable from a complex and organised society or, as he succinctly put it, the relation between spontaneous and social order.

Within two years of reaching Manchester he had produced his small but pathbreaking book *USSR Economics.* It was the result of a flash of understanding of the kind which genius enjoys. He saw that the idea of rational planning of the whole of the economic system by Governments was a complete illusion. Then, with remorseless logic, he asked himself: if central economic planning is indeed a chimera, what is it that Governments which set out to engage in it, are in fact doing? And, prophet as he was, he foresaw the answers. In countries, such as Russia, where the ideas were then being ruthlessly pursued, the result would be the creation of a slave state. In other Western countries (and he had in mind among these Britain) where individual freedom was more deeply entrenched and tenaciously defended, the attempts of Governments at central planning would simply lead to great muddle, with increased public intervention in industrial investment, economic debility, widespread price controls and inflation.

This central discovery he filled out in his later writings. He sought to explain by word, and by an ingenious film, the subtleties and efficacy of free market operations. Admitting to weaknesses in a system of private enterprise he developed ideas, along Keynesian lines, for the prevention of mass unemployment and for the more active encouragement of technical advance. He displayed in novel ways how, as an industrial organisation grows in size, it tends towards inefficiency in operation. He argued for the crucial role of profits in a free economy: "There exists no fundamental alternative to the system of money-making and profit-seeking".

After 1948 his mind moved into more abstract and philosophical fields. But in a remarkably short time he had left behind him a set of writings which economists in the future will turn to and find rich and stimulating.

John Jewkes

Though Michael Polanyi wrote a number of studies in the philosophy of science, his contribution rests mainly upon a single work, *Personal Knowledge,* first published in 1958. In both style and content it ran strongly counter to the logicist trends of the time, and was not easily assimilated into professional philosophy of science, though it was greatly appreciated by many working scientists. Written in a limpid, urbane style, drawing upon a wider range of experience than was usual in philosophy of science, it might be properly thought of as setting a trend towards 'science criticism', bearing a somewhat similar relation to science that literary criticism does to literature.

Two philosophical themes dominated this work, and all Polanyi's reflection on the nature of science; the theme of 'tacit knowledge' and the theme of the analogy of perception. Polanyi never tired of emphasising in many contexts that we know 'more than we can tell', a truth not only of the practical arts but as he persuaded many of his readers, of the intellectual arts as well. Explicit scientific knowledge rests for its intelligibility and grounding upon an undetermined basis of tacit knowledge, which if made explicit rests again upon its own basis of the tacit. Explicit knowledge is public knowledge, but tacit knowledge is private, and both sorts of knowledge are encompassed within science.

The relation of explicit knowledge to its penumbra was to Polanyi either identical with or perhaps only similar to the relation between focal and subsidiary awareness in perception, attending from something to something else. He elaborated this metaphor in a number of works.

That science was a part of society, that it was within the larger society a society itself, but a society with a peculiarly morally demanding mode of being, was both exemplified by the man himself, and the central message of his philosophical work.

Rom Harré

announcements

Appointments

Dr Peter W. Likins, of the University of California, Los Angeles has been named dean of Columbia University's School of Engineering and Applied Science.

Dr Michael A. Moore of Sussex University to Professor of Theoretical Physics at the University of Manchester.

Dr R. J. Ellis to a personal Professorship of Biological Sciences at Warwick University.

Dr Hugh Burkhardt to Professor of Mathematical Education at Nottingham University.

Sir John Habakkuk, Vice-Chancellor of the University of Oxford, has been elected Chairman of the Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom for the year 1976–77.

Awards

Professor N. Kurti of the University of Oxford has been made a Chevalier de la Légion d'Honneur.

Professor C. T. Dollery of the Royal Postgraduate Medical School has been made a Chevalier de l'Ordre du Mérite.

Dr Charles Elton of the University of Oxford, **Dr Rene Dubos** of the Rockefeller University and **Dr Abel Wolman** are the recipients of this year's Tyler Ecology Award.

International meetings

27–28, **The Handling of Toxico- Information,** Bethesda (Dr A. Wykes, Toxicology Information Program, National Library of Medicine, 8600 Rockville Pike, Bethesda, Maryland 20014).

Person to Person

Information is required on national and international efforts to popularise science, on famous science writers from all over the world and on awards given to science writers. Scientists who communicate to laymen please send their opinions to Zaka Imam, Assistant Editor, Science Reporter, Council of Scientific and Industrial Research, Rafi Marg, New Delhi 110001, India.

Astronomer attending international meeting wishes to exchange 3-bedroomed house (sleeps 6 and baby) in Sussex for house or flat (sleeping 5 and baby) near Grenoble, France, for one month during August 1976 (until about 3rd September—meeting ends on 2nd). Enquiries to Dr R. C. Smith, 38 The Avenue, Lewes, Sussex BN7 1QU, England.

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

June 2–4, **Frequency Control,** Atlantic City, N.J. (Dr John R. Vig, USAECOM, ATTN: DRSEL-TL-MF, Ft. Monmouth, N.J. 07703).

July 17–22, 1977, **Macromolecules,** Dublin (Symposium Officer, Macro Dublin 77, Institute for Industrial Research and Standards, Ballymun Road, Dublin 9, Ireland).

September 6–9, **Structure and Kinetic Approach of Plasma Membrane Functions,** Grignon, France (Deadline for application: May 15) (Dr Alain Paraf, Station de Virologie et d'Immunologie, Route de Thiveral, 78850 Grignon-Thiveral, France).

September 22–23, **Laboratory Animal Housing,** Washington DC (E. W. Grogan, Executive Secretary of ILAR, National Research Council, 2101 Constitution Avenue, N.W., Washington, DC 20418).

September 22–24, **Congress of the European Chemoreception Research Organisation,** Reading, UK (Deadline for registration: June 30) (Dr G. H. Dodd, Department of Molecular Sciences, University of Warwick, Coventry CV4 7AL, UK).

October 11–15, **Structure and Uses of Biopolymers** (Professor Alan G. Walton, Department of Macromolecular Science, Case Western Reserve University, Cleveland, Ohio 44106).

October 18–20, **Platinum Co-ordination Complexes in Cancer Chemotherapy,** Dallas (Deadline for abstracts: June 30) (Third International Symposium on Platinum Co-ordination Complexes in Cancer Chemotherapy, Wadley Institutes of Molecular Medicine, 9000 Harry Hines, Dallas, Texas 75235).

November 9–10, **Techniques for the Retrieval of Chemical Information,** London (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN, UK).

nature

May 13, 1976

What follows the squabble?

THE engineering profession in Britain has been in disarray for some time over its structure. There are fifteen separate institutions, each of which fulfils the double role of acting as a learned society and looking after the professional interests of its particular members. Ten years ago the Council of Engineering Institutions (CEI) was formed as the first move towards providing some sort of unified body that could represent engineers to government, in international circles, or just to each other; there was never any intention that the institutions should lose their separate identities as a result of these moves.

Unfortunately, the CEI has turned out to be just what its title says it is—an assemblage of institutions, each with its own pride, prejudice, president, executive secretary, council and awareness of the size of its membership, be it above or below the average. Thus the small institutions have been worried about being steamrollered in the CEI, while the big ones have been worried that they can be outvoted. As a result, progress towards establishing a real voice for the engineering profession as a whole has been slow and marked by considerable bitterness which led late last year to the Institution of Electrical Engineers (one of the biggest institutions) giving a year's notice of intention to quit the CEI.

Maybe this threat has had a salutory effect at a time when the CEI's statutes are now being prepared for approval by the Privy Council. In the months of negotiation ahead compromises are on the cards all round, in the knowledge that the Privy Council is hardly likely to endorse a constitution to which at least one major institution objects (and the mechanical engineers seem to be equally unhappy).

Engineers can ill afford the time and emotional, even conspiratorial, energy that is being spent by their representatives in arguing over the CEI. If conciliatory gestures fail in the near future it is inevitable that demands for a public enquiry into the organisation of the engineering profession will grow, and with good cause. There are obvious attractions to handing the whole mess over to a small committee whose members have no axe to grind, and gracefully accepting their solution. But if this is done, it is important that the terms of reference of the public enquiry should be right. What is really needed is not a convenient way of patching together a group of institutions by neatly balancing voting procedures and carefully avoiding putting a bunch of president's noses out of joint. The body that should be created is a Council of Engineers to which individuals would have a stronger sense of loyalty. Some argue that modelling such a council on the General Medical Council would be a good thing, producing a lean organisation with some lay membership, concerned with qualifications, discipline, education and so on. Certainly some bread-and-butter matters such as the nature of engineering qualifications and their interchangeability within the European Economic Community need attention, and such a body would devote much of its time to these affairs. But engineers should not pass up the opportunity to go further and use the council to provide an accessible profession-wide forum.

Engineers are often not people with the broadest vision, indeed sometimes their vision seems narrower even than that of scientists. In times of plenty this may not seem to matter too much—there will always be work available and few will question the benefits of technology. The problem surfaces only when times are hard, and it may be characterised as a loss of nerve. Technology is then not only an easy target for cutbacks but is accorded the blame for many of our woes. On the whole engineers do not seem to have come to terms very well with new moods in society, and one of the reasons for this is probably that there is not a great enough number of them prepared to read, think and talk together about the place of the engineer in society. This surely must be done on as wide a basis as possible, using the resources that all sectors of the profession can bring. While there is dissent about the CEI it cannot be done effectively. A public enquiry should at least be aware that some needs of the engineering community go beyond those of simply voting, acquiring a string of qualifications and doing a day's work. ☐

The difficulties facing West German science

What is the current state of science in West Germany? **Rainer Flohl** reports on a situation which scientists in other developed countries may find familiar

SCIENTIFIC research in West Germany is going through a difficult period. It is suffering from financial restrictions similar to those in many other countries. More importantly, it has been affected by the structural changes in West German universities over the past few years. Indeed, after a belated advance in the mid-1960s, when it received increased government support, West German science again stands on the brink of disaster. The winner of the Nobel Prize for Chemistry, O. E. Fischer of the Munich Technical University, has asserted that West German universities are losing ground in international terms. He compares research units in West German institutions of higher learning to empty shells, and the strong teams in Anglo-Saxon countries to battleships. German scientists cannot hold their own against this kind of opposition: the once-successful *Geheimratsystem* has disappeared, and with it has gone the glamour which had attracted scientists to West Germany from all over the world.

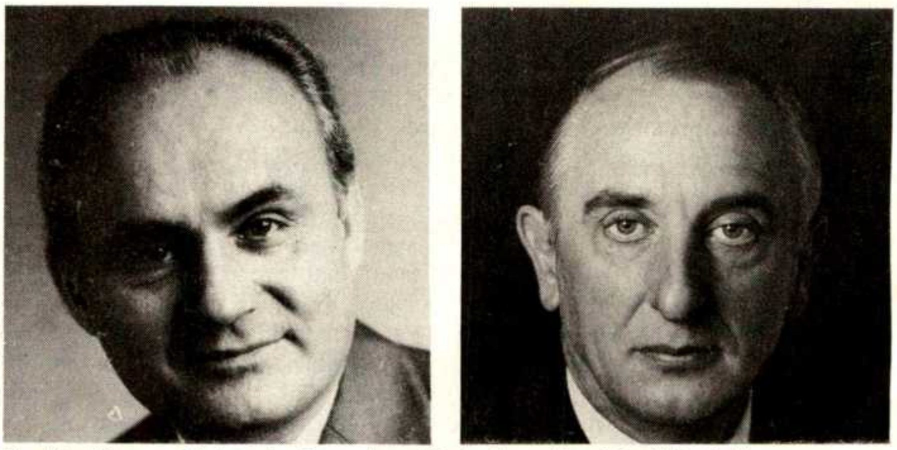
The overthrow of that system was in fact a reaction to the growing demands of an ever more complex science. The anticipated consequences—intensified cooperation among scientists, greater "teamwork", more criticism and self-criticism—have failed to materialise. But this failure was disguised in the early 1960s when increased financial support for science led to a certain amount of progress. Now the deterioration is apparent, though the true situation is not generally understood. There are three reasons for the deterioration: the aforesaid structural change; the overburdening of universities with teaching; and the financial crisis, which has drastically exposed the weak position of science.

The overburdening with work of teachers in higher institutions is the result of the explosion of further education, which even now continues to attract more and more students to universities. Growing numbers of university teachers were needed to instruct the students. These teachers wished not only to teach, but also—in the German tradition of "unity of research and teaching"—to carry out research for themselves. This has led to a doubling of the number of scientists in the Federal Republic over the past few years. The means for research work have not risen proportionately, so the average research worker has less money than at any previous time. Besides this, the lecturers have had to spend more time on their teaching. Only in this way could they keep up with the rising number of students, whose education—for political reasons—appears to be more important than research work. The time available for research has been reduced because the democratisation of higher education demands too many meetings and additional administrative expenses.

The financial crisis has led to a drastic reduction in the funds available for research. Many programmes have had to be arbitrarily curtailed. In 1975 approximately 30 to 40% of allocated funds were withdrawn; there will be no change in the situation in 1976. People have accepted the change without complaining. Unrest at the universities has subsided. The only remaining tensions are in the departments responsible for teacher training; but fully qualified students have increasing difficulty in finding work, which makes them insecure. Many of them blame the so-called *Radikalenerlass* rather than their own poor marks for their failure. The *Radikalenerlass* was an administrative regulation designed to prevent professed enemies of the constitution—in particular members of the Communist Party—from getting jobs in the public service. Many rumours arose because of the generally clumsy and bureaucratic methods used to check public service applicants. It was said, among other things, that a signature on a pamphlet was sufficient to get an applicant rejected. The regulation has recently been abolished, or rather administered in a different manner.

The Federal Republic has encountered these problems at a time when there were justifiable hopes of improving the effectiveness of scientific research. The two most important organisations conducting pure research in the Federal Republic—the German Research Organisation (DFG) and the Max-Planck Institutes (MPG) — had elected new Presidents who were well-qualified and open to new ideas for reform. Professor H. Maier-Leibnitz, President of the DFG, which receives about DM600 million a year from the Federal and State governments for the furthering of basic research, has a wealth of experience and success as a practical scientist: he built the Federal Republic's first experimental reactor in Garching near Munich, and it was due to his initiative that the Franco-German high flux neutron reactor was later erected in Grenoble. Not least because of suggestions from Professor Maier-Leibnitz, both instruments became focal points of research, and he became an international figure through his contribution to neutron optics. The President of the Max-Planck Institute, Professor Reimar Lüst, is also a physicist. Formerly President of the European Space Research Organisation (ESRO) and Chairman of the German Science Council, Lüst was the first man to use barium clouds for the discovery of astrophysical phenomena. The Max-Planck Institute also receives DM600 million from the Federal and State governments from funds set up to finance independent institutions.

Only time will tell whether Professor Maier-Leibnitz and the German Research Council will manage to restore science to its former position and thus unlock the creativity which is essential for successful research. The Max-Planck Institutes' first priority is to protect scientists from the dead hand of the bureaucracy. But both institutions face exceptional problems,



Photos: PIB, Bonn

The West German Minister for Research and Technology, Hans Matthöfer (left), and the Minister for Education and Science, Heinrich Rohde

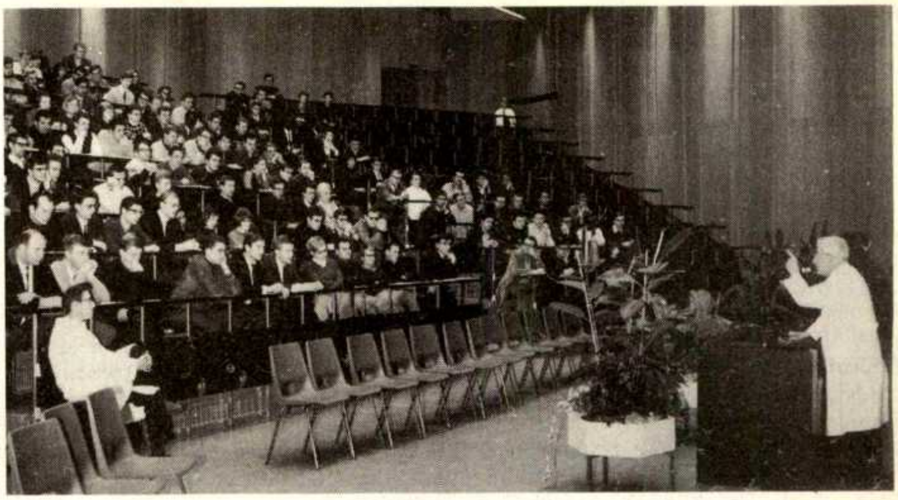


Photo: PIB, Bonn

Students at Saarland University: for them, what sort of future?

especially as the "reform" of the universities has blocked posts for the new generation of scientists, however well-qualified, since large numbers of mediocre young scientists secured life-long tenure in earlier years.

At the beginning of April the German Research Council published its *Grauen Plan* ("Gray Paper") analysing the present state of research in the Federal Republic. From its assessment of the present situation, and the many obvious deficiencies thus revealed, it derives suggestions for improving research, though it accepts that not all the ideas it puts forward can be carried out. The Paper covers the years 1976 to 1978, and is the most detailed and honest document yet compiled about German research. The main section deals with the structure of the universities and the financing of research. For the first time, moreover, scientists from universities were consulted in the drawing up of the document: out of 1,300 scientists approached for their views on the state of research in universities and the condition of their own field of work, 1,000 responded. The results of this inquiry are also included in the Paper, which in parts is more a catalogue of inadequacies than the report of an enquiry, though it should not be overlooked that particular institutes are in fact in the lead internationally.

To overcome at least the financial stagnation of research, Professor Maier-Leibnitz called upon the Ministers of Culture and Science in the Federal Republic and its component States to increase the capacity for work in the country's universities, by promoting and supporting important research and at the same time making use of existing but insufficiently used research facilities. In this way, with less resources, a considerable improvement in quality could be achieved—something which had been greatly neglected during the continuous expansion of higher education. An increase in the budget of 10% (DM60 million) would be sufficient to begin with, which amounts to only ½% of the DM13,000 million which is spent annually on higher education in the Federal Republic.

In this connection it must soon be clarified whether the balance between applied research and pure research is to be maintained in Germany. The former has expanded rapidly over the past few years through government grants. Those private institutions entrusted with the furtherance of science have so far refrained from open criticism of this development. To understand the problem it is only necessary to consider the vast sums which have been spent on applied research. Whereas the state granted DM850 million in 1974 to further general research in universities and private institutions, it allocated DM3,200 million for advanced programmes of applied technology (nuclear research and technology, space research and technology, data processing, marine research and other technology).

Criticism of the disproportion between applied research and pure research may be justified when it is considered that Germany is living on the results of past research, without worrying about the future. No politician seems to give serious consideration to the scientific source for the technology of the 1980s. This behaviour is reminiscent of the carelessness with which people treated questions of raw materials and the environment until recently. Many scientists are worried about the situation: it puts the Federal Republic at a disadvantage internationally; it also threatens those industrial nations which are short of raw materials, whose economies depend on the progress achieved in their laboratories.

The final decision on the relationship of pure research to applied research lies with the political authorities. They have never shown any special enthusiasm for pure research, which has little propaganda value for them in comparison with basic technological projects. Publicity and politics are not, as is often claimed, the enemies of science; they are simply indifferent to pure research. The "Gray Paper" ought to bring about a change of attitude. But will any of the politicians read it? □

The state of health of NIH

Colin Norman in Washington looks at the National Institutes of Health, just investigated by a Presidential Commission

ON various occasions in the past few years, the National Institutes of Health (NIH), one of the largest and most prestigious biomedical research centres in the world, has been beset with personnel and budgetary upheavals. Two NIH directors have been fired, ostensibly for political reasons, repeated clashes between Congress and the Executive branch over the overall health budget have frequently thrown planning at NIH into utter confusion, and many of the agency's programmes have been suffering from chronic shortages of cash while others have been receiving relatively generous support. It has not been a very happy place.

The problems which have plagued NIH and, by extension, the universities and medical schools whose work NIH supports, stem from a variety of sources. They range from the fact that NIH's budget has climbed to more than $2,000 million a year, which makes the agency politically very visible, to false public hopes that medical science can produce swift solutions to complex health problems. But the chief irritant is clearly the 1971 National Cancer Act.

The instrument which officially launched the federal war on cancer, the act abruptly changed many established methods of supporting, planning and managing biomedical research. It also elevated the National Cancer Institute (NCI) to a privileged position in NIH, and caused vast sums of money to be poured into cancer research while other areas of biomedical research have been growing slowly, if at all. But the Cancer Act, more than any other single factor, has sharply focused political attention on NIH's affairs.

Early in 1974 when morale at NIH was at a low ebb, following some particularly severe upheavals, Congress, at the instigation of Senator Edward M. Kennedy and ultimately with the support of the then Secretary of Health, Education and Welfare, Caspar Weinberger, passed a bill establishing a Presidential Commission to investigate NIH's affairs and make recommendations for relieving some of the strains. Chaired by Franklin D. Murphy, a former medical school dean who is now chairman of the Times Mirror Corporation in Los Angeles, the panel conducted an exhaustive 15-month inquiry, taking evidence from scores of witnesses and commissioning several independent studies of key aspects of federal health policy. It submitted its long-awaited report to President Ford and Congress on April 30.

A plea for more stability in funding, and for NIH to be left under the control of scientists rather then politicians, the report with its voluminous appendices offers few radical suggestions for changing NIH's operations. Its central message is summed up in the introduction : "The United States can take pride in a remarkably productive biomedical and behavioural research effort. The panel is convinced that despite the appearance of strains in the structure and some dislocation in the parts, the edifice is sound".

Though some people have been quick to criticise the report as being a self-serving document (five of the seven panel members were drawn directly from medical schools), it nevertheless offers some constructive analysis of NIH's activities. Perhaps its most far-reaching and controversial suggestion is that a tentative stem should be taken to re-integrate the National Cancer Institute into the rest of NIH. This should be accomplished, the panel suggests, by re-constituting a powerful committee which now oversees the working of the National Cancer Act into a more broadly based body with jurisdiction over the entire NIH effort.

To understand why such a seemingly mild suggestion is controversial, it is necesary to appreciate some of the history of the National Cancer Act and present cancer politics. The act stemmed largely from the recommendations of a blue-ribbon panel of scientists and lay members chaired by Benno C. Schmidt, a New York industrialist with influential political connections and a persuasive manner. The panel, which reported in December 1970, argued that NCI should be separated from the NIH bureaucracy, be given a separate budget and that its director should be appointed directly by the President. An independent status, the panel argued, would give cancer research greater visibility and enable NCI to adopt a more sharply focused research effort.

The plan was fought at the time by many scientists who were alarmed at the prospect of separating cancer research from closely related fields of study, and when Congress passed the National Cancer Act, it kept NCI within NIH, but only just. The NCI director is appointed directly by the President, and the NCI budget is drawn up and submitted separately from the budgets of other NHI institutes. The Act also established a powerful, three-member President's Cancer Panel to oversee the working of the act and to provide an extra link between NCI and the White House. Benno Schmidt was appointed chairman of the Cancer Panel. He has since devoted much of his time and considerable talents to his duties.

Since passage of the Act, the NCI budget has grown swiftly, though not as rapidly as the Act envisaged. Between 1970 and 1975 NCI's budget rose by 280%. In the same period, the budget for the National Heart and Lung Institute doubled (research on cardiovascular diseases was given a boost by a bill passed by Congress in 1972), but the budgets for all the other eleven institutes combined rose only by 20%. Cancer's share of the total NIH budget climbed from 16.6% to about 35%. At the same time, NCI began funding a growing share of its research activities by contracts rather than grants, a move which created some discontent in the biomedical research community.

Considerable resentment has been expressed at the privileged position of NCI, and the fact that the growth in support of cancer research has seemingly been made at the expense of other less politically favoured areas of research. Nevertheless, the cancer budget has considerable popular support and is defended by a powerful coalition of politicians, cancer researchers and others, not least of whom is Benno Schmidt. It would be politically difficult simply to merge NCI back into NIH, which is why the panel is very cautious in its recommendation.

It begins by noting that it "both recognises and supports the priority for cancer research established by the Congress", and suggests that the special status of NCI be retained "at this time". It recommends, however, that the President's Cancer Panel be reconstituted by statute as the President's Biomedical Research Panel, with responsibilities extending over all functions of NIH. Its membership should be broadened, it should provide a link between NIH and the White House, and offer advice on the total biomedical research effort. Finally, the panel suggests that "this proposal provides the opportunity, if experience so dictates, to fully integrate the national cancer programme with the other programmes of NIH in due time". The panel's report notes that Benno Schmidt, who was one of the seven panel members—further testimony to his influence over biomedical research matters—did not participate in decisions leading to this recommendation.

Panel proposals

THE following are among the more important recommendations of the President's panel.

- The President's Cancer Panel should be reconstituted and its responsibilities should extend over all NIH programmes.
- A new NIH advisory board should be established to provide advice to the Director of NIH and a measure of public input into NIH policy.
- No new research institutes should be established, and consideration should be given to amalgamating some existing institutes.
- The research programmes of the Alcohol, Drug Abuse and Mental Health Administration should be strengthened, but the panel decided, by a 4 to 3 vote, to recommend against transferring them to NIH.
- Investigator-initiated grants, rather than contracts, should continue to be the chief means of supporting research by NIH.
- The Director of NIH should have at his disposal a contingency fund of not more than 1% of NIH's budget, to initiate new studies.
- Legislation should be passed to preserve the secrecy of peer review deliberations and to protect the confidentiality of grant proposals until they have been funded.
- NIH should resist pressures to participate in long-term patient care.

The panel members were: Franklin D. Murphy, Times Mirror Corporation (Chairman); Ewald W. Busse, Duke University Medical Center; Robert H. Ebert, Harvard Medical School; Albert L. Lehninger, Johns Hopkins University Medical School; Paul A. Marks, Columbia University College of Physicians and Surgeons; Benno C. Schmidt, J. H. Whitney and Co.; and David B. Skinner, University of Chicago Hospitals and Clinics.

The proposal is likely to be controversial for several reasons. First, many scientists who have been resentful of the privileged position and burgeoning budget of the cancer programme will not be happy to see the panel responsible for much of that growth placed in charge of the rest of NIH. In that regard, however, it should be noted that while Schmidt has skilfully fought for the interests of the cancer programme, he has also spoken out on many occasions against letting NCI's budget grow at the expense of other areas of NIH. A second factor is, however, potentially more disturbing. The establishment of a powerful committee to oversee the programmes of NIH, though it will have no direct executive power, could seriously undermine the authority of the NIH Director. Though the present incumbent, Dr Donald S. Fredrickson, said in an interview last week that he would willingly work with such a panel if it is established, he would clearly not relish the thought of there being two Popes in charge of NIH and finding that he is the one in Avignon.

Another major point which the panel addresses is the confusion introduced into NIH planning by the repeated scraps between Congress and the Administration over the budget for the Department of Health, Education and Welfare, of which NIH is a part. Every year, Congress approves a larger budget for HEW than the president requests, and the matter becomes a political football with vetoes, budget deferrals and so on clouding the picture. Consequently, NIH frequently doesn't receive its budget until the fiscal year is nearly over, and it doesn't know until the last moment how much money is going to be available.

Fredrickson acknowledged last week that such uncertainties are a "very serious problem for NIH". But it is difficult to see a solution. Certainly, the panel's recommendations are unlikely to be accepted. The panel, in short, suggests that Congress and the Executive Branch should approve a new budgeting system for NIH which would allow forward funding of multiyear grants and contracts through a single appropriation instead of the present system under which new budgets must be approved each year. That system would, however, introduce more inflexibility into the federal budget, and would thus run counter to the desires of this Administration. The proposal, moreover, could just as easily apply to the rest of the Federal Government's $20,000 million-a-year research budget, so the Administration would be wary of setting a precedent.

Finally, it should be noted that the strains within NIH and the rest of the biomedical research community have been less apparent in recent months. In part, that is due to the introduction of some new policies, such as a new granting arrangement in NCI to replace some contracts, and improvements in the quality of peer review of contract proposals. It is also due in part to the appointment last year of Fredrickson as NIH Director. A skilled administrator who has earned the respect of both scientists and politicians, Fredrickson has had a considerable calming influence. Nevertheless, many of the fundamental problems which have led to discontent in the past remain, and it is unlikely that the panel's pitch for more independence for NIH will solve them. □

USA

Human rights guidelines adopted

Colin Norman reports on moves in the United States to combat the repression of scientists

FOLLOWING a recent spate of complaints that it has been too timid in speaking out against violations of human rights, particularly those of scientists, the National Academy of Sciences has adopted a set of guidelines to govern its actions when future infringements are brought to its attention. One of the Academy's most outspoken critics on such matters, Dr Jeremy J. Stone, Director of the Federation of American Scientists, last week welcomed the guidelines as offering a "constructive approach".

Proceeding from the observation that "violations of human rights . . . occur in many—perhaps all—countries", the guidelines suggest that the Academy should try to battle such violations by "persuasion on moral humanistic grounds". But they add that this approach "may require fortification by stronger measures".

The Academy operates for the US government a number of scientific exchange agreements with other countries, which gives Academy officials considerable contact with their foreign counterparts. In the past, the Academy has used such contacts to make private, face-to-face representations on behalf of beleaguered scientists, particularly repressed Soviet intellectuals. But only in the case of Andrei Sakharov has the Academy issued a public protest, and a number of scientists, both inside and outside the Academy, have recently criticised it for not speaking out more forcefully on other occasions.

The guidelines state that the Academy will continue to remonstrate influential officials in other countries, choosing individual cases of scientists and engineers to protest. "We will continue to use the quiet informal contact as our principal mode of communication with peer groups and governments in other countries", the guidelines state, but they add that "we do not eschew entreaty by public vehicles; indeed, we anticipate that such action will occasionally be appropriate". The strongest leverage which the Academy has in such negotiations is the threat of withdrawal from participation in exchange agreements, and the guidelines recognise that such a measure is available. Indeed, "it is always implicit in the background, but (it is a measure) that we can use only rarely".

In a related development, Academy members adopted a resolution at the annual meeting last month affirming principles of freedom of inquiry and expression. They urged colleagues around the world to join them in sending the following (or similar) signed statement to the Academy:

> I hereby affirm my dedication to the following principles.
>
> ● That the search for knowledge and understanding of the physical universe and of the living things that inhabit it should be conducted under conditions of intellectual freedom, without religious, political or ideological restrictions.
>
> ● That all discoveries and ideas should be disseminated and may be challenged without restriction.
>
> ● That freedom of inquiry and dissemination of ideas require that those so engaged be free to search where their inquiry leads, free to travel and free to publish their findings without political censorship and without fear of retribution in consequence of unpopularity of their conclusions. Those who challenge existing theory must be protected from retaliatory reactions.
>
> ● That freedom of inquiry and expression is fostered by personal freedom of those who inquire and challenge, seek and discover.
>
> ● That the preservation and extension of personal freedom are dependent on all of us, individually and collectively, supporting and working for application of the principles enunciated in the United Nations Universal Declaration of Human Rights and upholding a universal belief in the worth and dignity of each human being."

An Academy official said last week that the Academy will act as repository for such statements and make public the response. Confidentiality will be guaranteed to those who request it.

Signed statements should be sent to the National Academy of Sciences, 2101 Constitution Avenue NW, Washington DC 20418. □

COMECON

As a result of the recent meeting of the Comecon Executive in Moscow, coordination of economic plans will provide the chief means of cooperation for the Comecon countries between now and 1980. A programme for this integration produced last June includes specialisation and cooperation on key scientific and technological problems, and a strengthening in the energy, fuel and raw materials sectors.

One of the main fields of cooperation is atomic power engineering. According to the Joint Forecast for the Development of Nuclear Power Engineering in Comecon for the period up to 1990, the combined capacity of atomic power plants in the Comecon countries by 1980 will be some 30 million kW. As with most Comecon projects, the Soviet Union plays the leading role in such developments, but the contribution of the other member countries to joint research and the exchange of nuclear technology is stated to be "constantly increasing". In 1975 this mutual exchange is said to have increased by 23%. Though no precise basis for this figure is specified, an increasing amount of work in this field is apparently being carried out beyond the Soviet frontiers.

Thus a joint investigation into nuclear breeder reactors by the Institute of Nuclear Research (Swierk, Poland) and the Institute of Nuclear Power of the Byelorussian Academy of Sciences is being carried out at Swierk, using the *Maria* reactor. Some bipartite agreements in this field have also been signed between individual member countries. These include a programme of cooperation between the Czechoslovak and Hungarian Atomic Energy Commissions covering reactor physics, the development of nuclear instruments and the development of nuclear power stations. Talks between J. Neumann and Gyoergy Osztrovszki, the Commission Chairmen, included discussion of the Heller-Forgo system of air-condenser cooling for reactors. Similar bilateral discussions have also been held between Bulgaria and Rumania.

The Conference of Comecon nuclear power engineers, which met earlier this year in Rheinsberg, East Germany, made no absolute claims for future expansion: the share of nuclear energy in the growth of electrical energy in the Comecon bloc over the last five years was 17%, it was said, and this "should" increase to 27% in the next five years. The general picture, however, is one of optimism. Poland is introducing a special training scheme for nuclear engineers at the Cracow Academy of Mining and Metallurgy, using a 100 kW nuclear reactor provided by Swierk, and the Soviet Union is expanding the field of cooperation outside the Comecon block, assisting the Finns in the construction of a nuclear power station at Loviisa.

Cancer research provides another major field of cooperation. A conference in March of this year, held at the National Institute of Oncology in Budapest, laid down guidelines for future cooperation and integration. Hungary is to be in charge of pharmaceutical research and the testing of drugs, and the Soviet Union, notably the Moscow Oncological Institute, is to continue its research into the feasibility of neutron therapy, in which it is claimed that "substantial progress" has already been made. A Comecon X-ray morphological and diagnostic laboratory has been opened in Obninsk, and the possibilities of using π-mesons and proton radiation for the treatment of carcinomas is under consideration. This line of research, it is postulated, could be carried out with the cooperation of the Dubna Nuclear Research Institute.

The conference considered it particularly important to extend its contacts with the international cancer research organisations. WHO, the International Oncological Union, and the European Cancer Research Organisation already have links with certain Comecon States; the new proposals, however, suggest information-sharing between these bodies and the Comecon programme as a whole, presumably through the Moscow Oncological Institute, which acts as a coordinating centre and clearing-house for Comecon cancer research.

- One promising outcome of the recent agricultural crises in the Comecon block is the availability to the USA of credits from the sale of "agricultural surpluses" to Comecon countries. In the case of Poland, some of these credits are to be used to establish the "Maria Sklodowska-Curie" fund to finance joint research, the exchange of specialists and the organisation of conferences, thereby providing a fresh stimulus to Polish–US scientific contacts. Recent joint undertakings between the two countries include a joint expedition to the North-West Atlantic to study plankton resources, and hence the possibility of developing new fishing grounds in the area.

- At a meeting in Luxembourg in February of this year, Gerhard Weiss, the Chairman of the Executive Committee of Comecon, and Gaston Thorn, the President of the Council of (Prime) Ministers of the European Community, discussed a number of fields of cooperation between Comecon and the EEC, including standardisation, conservation, and statistics. Regarding conservation, the Comecon countries and Yugoslavia have now affirmed their willingness to see closer cooperation with the EEC. This emerged at a symposium in Dresden on technical questions relating to production processes with little or no waste products. Dr Hans Reichelt, the East German Minister for Environmental Protection and Hydroeconomy, welcomed Mr Brezhnev's proposal that an all-European Congress on cooperation in environmental protection should be held in the near future.

- The Royal College of Psychiatrists in the UK has adopted a resolution reiterating its condemnation of the abuse of psychiatry for political purposes in the USSR, and has pledged itself to do all in its power to secure the passing of a similar resolution by the World Psychiatric Association at its next World Congress in Honolulu in 1977. The proposed resolution runs counter to the expressed opinion of Dr Denis Leigh, the Secretary General of the WPA, who would prefer that all resolutions should deal with general ethical principles and avoid questions connected with religion, national policies, political belief and so on. He told the English-language journal *Soviet News* in December that he felt it important that the Soviet Union should be represented at the Honolulu Congress, and described the Soviet Society of Neuropathologists and Psychiatrists as "one of the most loyal" members of the World Psychiatric Association.

Vera Rich

SWEDEN

Science from the top

Wendy Barnaby reports from Stockholm on the International Foundation for Science's aid programme.

Few activities can cause such disillusion as the giving of foreign aid. Programmes hailed initially as life-savers often peter out with the sponsor complaining of lack of cooperation by the receiver, who in turn accuses the sponsor of paternalism or outright exploitation. Often the least satisfactory outcomes seem to bedevil the wealthiest sponsors. Money means organisation and, *ipso facto*, the diversion of creativity away from aid-giving into the maintenance of the organisation itself.

If wealth tends to bring problems, the International Foundation for Science (IFS) should on the face of it have more success than most aid organisations. Its total revenue for 1976 is expected to be Sk3,560,000 (about $790,000). The organisational side of the IFS looks healthy, too: it was formally established at a meeting in Stockholm last September as an afterthought to the initiation of some 45 projects. The only full-time employees are five administrators.

Each project is actually a research grant given to a young scientist from an underdeveloped country on the condition that his or her work is relevant to the needs of the country and carried out in it or some other underdeveloped land. The grants are given annually and are renewable for four years. The original intention behind the scheme was to try to stop the brain-drain of young African, Asian and Latin American scientists to developed countries because of the paucity of equipment and contacts at home. The grants—there are now 100 of them—have all so far been given within the broad field of biology applied to food production.

This encompasses six specific areas. The first is the development of local fish-farming through research on fish, of which an example is a grant of $13,000 given to a project in Nigeria's Federal Department of Fisheries to study the distribution of the popular cat-fish in order to collect fingerlings for stocking ponds. The second area is the improvement of small animals (mainly goats and sheep) of domestic and local importance—for example the research being done at the National Agricultural University in Lima to use agricultural waste products as more ficient food for guinea pigs, which are the main source of animal protein for the poor population in the upland region of Peru. The IFS has contributed $4,600 to the project. It also supports work on the morphology, physiology and pathology of vegetables, tubers, pulses and forage crops, such as that at the University of Malaya in Kuala Lumpur, to study the protein values of locally grown leaves, stems and tubers from cassava. This study—to which the IFS has given $2,300—is aimed at improving cassava for direct human consumption or for the production of leaf protein concentrate.

Studies of mycorrhiza (the symbiotic association of a fungus with the roots of a seed plant) in connection with afforestation form the fourth area of interest. At the Forest Products Research Institute, Kumasi, Ghana, research is being done into the most suitable fungi and the performance of field innoculation of pines with mycorrhizal fungi, the mass production of suitable fungi and the performance of the innoculated pines. The results will, it is hoped, contribute to Ghana's re-afforestation programme, and the IFS has supported it with $13,000. The fifth area is the improvement of traditional food fermentation processes—for example, a project under way at the University of Ain Shams in Cairo, Egypt, in which the waste materials from industrial food processing are being examined to see if they are suitable for controlled microbial growth which could lead to the economic production of protein. The IFS has given the project $7,000. The investigation of organic compounds from natural products traditionally believed to have medicinal applications is the sixth area. Here, the IFS has for example given $6,300 to research at the Haile Selassie I University, in Addis Ababa, Ethiopia, into the structure, properties and possible toxicological effects of a local plant used against tape-worm infections.

The fact that the grants are given in so many different places is not the only international aspect of the IFS. The funds are also contributed from many countries, mainly through national academies or other bodies representative of natural or social science or technology. In 1976, Sweden is expected to contribute just over half the total budget, Canada nearly one-fifth, and France, Belgium, the Federal Republic of Germany, the Netherlands and Japan will make up the rest between them. The President of the organisation—and the moving force behind its inception—is Swedish Professor Sven Brohult.

The specific projects mentioned so far illustrate the IFS pattern of a small grant being given to an individual scientist already employed at a research institute in an underdeveloped country. The organisation's involvement does not end there, however. Applications for grants in each of the six areas of interest are perused by scientific advisory groups, who recommend on their adoption to the Grants Committee. The advisers (who work for the IFS voluntarily) are scientists employed on research within the area whose applications they judge, and travelling in the course of their work to the regions in which projects are being carried out. In this fashion, the IFS is building up a network of contacts between researchers working in the same field in neighbouring countries who were not previously aware of each other's existence.

The IFS is so far committed to the potential of developed countries' science for aiding underdeveloped countries, a commitment which grew naturally out of its attempt to stop the brain-drain and one which can be strongly defended given the fact that so many researchers from underdeveloped countries have already been educated abroad. With this commitment goes a "science from the top" approach: support of researchers educated in science working by traditional methods in the hope that their work will ultimately benefit the other sectors of society. This is less fashionable now than the "science from the bottom" approach: educating the masses to contribute to the improvement of their condition through science, as for example Chinese peasants reporting on signs they see in the fields of approaching earthquakes. Ideally, each approach should complement the other. In the meantime, however, the IFS is seen to be doing something worthwhile. ☐

Sven Brohult, IFS President, and Olle Edquist, former Executive Secretary

IN BRIEF

Sea conference ends

The latest eight-week session of the 156-nation Law of the Sea Conference ended in New York last Friday with several bitterly-debated issues still outstanding. When the next round of talks is convened at the same venue in August, subjects for further discussion will include the extent of national jurisdiction and sovereignty over economic zones, the rights of landlocked states, the most suitable form of disputes procedure, and freedom of access for scientific research—a particularly contentious area offering scope for espionage and mineral plunder, in the view of some third world nations. Nonetheless, the divergent views of the underdeveloped and industrial states have closed slightly at least on the issue of control over deep sea mineral exploitation, and a compromise solution seems within reach. As the conference ended the Soviet Union protested at recent US legislation to extend its fishing limits to 200 miles by March 1977. One view, however, is that the US move would hasten that similar unilateral action which other worried states would probably have taken anyway.

Fluorocarbon report delayed

A crucial report by the National Academy of Sciences on the possible effects of fluorocarbons on the ozone layer has been delayed by up to three months because of new data which suggests that the threat may not be as serious as first predicted. It seems that chlorine, liberated from fluorocarbons, in the upper atmosphere, may react with nitrates to form a moderately stable compound. If correct, the theory, which is backed up by some direct measurements, means that the reaction would provide a "sink" for destructive chlorine atoms, reducing their effect on the ozone layer substantially. The Academy report, which was in the final stages of drafting when the new data came in, has been delayed until July.

Monitoring the stratosphere

Spurred by the recommendations contained in February's US Government decision granting Concorde commercial access to the United States for a 16-month trial period, Britain and France have entered a joint programme with the US to monitor the stratosphere. Under the agreement, signed in Paris last week, the participants will aim to improve and extend monitoring techniques, and record long-term fluctuations in global ozone levels. The US recommendations followed concern that nitrogen oxides in supersonic aircraft exhaust might destroy stratospheric ozone through reactions like those involving fluorocarbons from aerosol sprays.

Mutiny on the Bounty

THOMAS H. JUKES

SCIENTISTS have laboured long and mightily in the past half-century to help farmers increase food production, and to identify and prevent nutritional deficiency diseases. Their efforts were so successful that a surplus of food piled up in the USA, although not in most other countries, and there was a great deal of overeating. Simultaneously, many infectious diseases ceased to be significant causes of mortality, as a result of public health measures, including immunisation, and the discovery of chemotherapeutic drugs and antibiotics. Deaths from cardiovascular disease, cancer, hypertension and other degenerative diseases went to the head of the statistical tables. It is an obvious and easy oversimplification to blame these on food because of their association with obesity, especially the cardiovascular group. But the fact that overeating leads to fatness is no reason for blaming the food rather than the gormandiser.

When the choice between guns and butter was rhetorically offered by Goebbels to the German public, little did we expect that, a few years later, butter would be placed with guns among the enemies of human beings. Yet *Nature* (April 15, page 565) wants every cow to carry a government health warning for making butterfat. The warning should be on the bathroom scales, not on the cow. The warning would be just as logically placed on every nubile woman, for human milk contains fats similar to those in cow's milk, and we are told that pre-arteriosclerotic changes can start at a very young age. I doubt that this suggestion will get very far.

In fact, when I was asked recently what foods were not under suspicion of being unsafe to eat, I could think of only one: sawdust. Fats, of course, are deadly; the saturated kind cause heart disease and cancer, and the unsaturated ones destroy vitamin E. Sucrose is belaboured as being addictive and diabetogenic, except, apparently, when it occurs in orange juice (5% sucrose) and pineapple juice (8% sucrose), or when it is brown instead of white. School children who eat candy bars are being reprimanded by adults who smoke cigarettes and drink vodka on the rocks. School authorities are shutting down the vending machines, so that enterprising pupils are bootlegging soft drinks.

We eat too much protein, of course, about twice as much as we need, and we had better feel as guilty as possible about this. The US House of Representatives just passed a bill to prevent the Food and Drug Administration from restraining the promotion of para amino benzoic acid as a vitamin, much to the joy of the "health food" stores. For if food makes you sick, vitamin pills must make you well, even non-vitamin pills that are only good for bacteria.

Food additives must go, say the consumerists, and the large companies follow suit by devising all-natural breakfast foods; nothing artificial added, no preservatives. Why let the big and growing market for "natural foods" go elsewhere? Sodium propionate stops bread from going mouldy, but it is artificial, even though it is normally present in cheese, so out it goes. Never mind the fact that some moulds produce carcinogens. Bacon is the villain, it contains nitrites, which form nitrosamines. However, more than two-thirds of the nitrites entering the average stomach come from saliva, and 86% of the nitrates, from which nitrates are formed in the mouth, come from vegetables.

Sawdust and bran alone remain, because we need more fibre to keep the bowels active, and thus prevent cancer. This is proved by the fact that there is very little cancer in Africa, where there is lots of movement. Oh, well! The English have always been preoccupied with that function, ever since the days of Beecham's Pills and Kruschen Salts.

Maybe we were better off when we counted nickels instead of calories.

correspondence

International exchange

Sir,—The fourth European Drosophila Research Conference met in Umeå Sweden, in the Spring of 1974. It was a very interesting conference, which was attended by more than 150 Drosophilists from all over Europe. However, a certain disappointment was apparent among many of my colleagues, because no Drosophilists from the Soviet Union attended.

The fifth Conference will be held in Louvain-La-Neuve, Belgium, at the beginning of next September. As the organiser of the conference I really hoped that, as well as the other European Drosophila geneticists, a delegation from the USSR could attend the conference. Therefore, at the suggestion of an American colleague, I wrote last July to Academician Belyaev, who is President of the Soviet Society of Genetics, in order to obtain from him a list of his colleagues who might wish to attend the conference. I received a list of about 20 names last September.

At the beginning of January, I sent a first announcement and preliminary registration forms to all of them. At the same time I wrote to the Chairman of the USSR Academy of Sciences, inviting officially a delegation from the USSR to attend the conference. I got some answers from the Drosophilists of the USSR who were eager to attend.

Recently, however, I received a very short letter from the foreign relations department of the Academy telling me that the scientists of the Academy do not plan to attend the conference. It is hard to believe that it constitutes a final answer, especially as the XIV International Congress of Genetics is supposed to be held in the USSR in 1978. Are we to understand that the exchanges between geneticists from West and East Europe are only one way?

F. Lints

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Birds on the Chagos Bank

Sir,—The recent communication from Hirons *et al.* (April 1, page 387) prompts this response. Their suggestions relative to rats and birds at Great Chagos Bank raise several questions. "Feral brown rats" (are those *Rattus norvegicus*?) are alleged to prevent full utilization of Eagle and Egmont islets by seabirds. The suggestion is made that " 'warfarin' and Liverpool virus" be utilized to remove the rats.

While many studies of island situations have indicated the predatory impact of rats on nesting seabirds, this is not a universal relationship. Our own studies at Enewetak Atoll in the Marshall Islands have failed to produce evidence of predation by rats (*R rattus* and *R. exulans*) on such colonies, which include one of more than 10,000 sooty terns.

Eradication of rats is difficult. If the rat colony is at all sizeable, the presence of the "warfarin" resistant gene is likely; and problems might well be encountered. I wonder whether the Liverpool virus is being confused with Salmonella bacteria, a now discounted mode of control. In this connection, the WHO caution against their use should be noted (FAO/WHO Expert Committee on Zoonoses, *Wld Hlth Org. techn. Rep. Ser.*, No. 378, 1967; WHO Scientific Group, Ecology and Control of Rodents of Public Health Importance, *Wld Hlth Org techn. Rep. Ser.*, No. 553, 1974).

This suggests that the terrestrial ecology of those islets should be adequately understood before drastic management efforts are attempted. Knowing more of the "recent surveys" would be most helpful.

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Cows and heart disease

Sir,—If a non-medico may presume to comment on coronary heart disease (Editorial, April 15), surely it is not the sacred cow that needs to carry a government health warning, but the golden car. Ignoring the nauseating exhaust products of the latter, I refer to the long term debilitating influence of car commuting. And not merely to confined physical inertia, but to the tremendous stresses involved in coexistence with one's neighbour within the relentless daily stream of traffic. To have a cyclist's eye-view in Boston, for example, is to see men possessed to a degree dangerously approaching that of mechanised Gadarene swine.

You say "Strangely, there is no evidence of a similar trend [of increasing incidence of CHD] for women". Is this because Equal Opportunity has not yet advanced far enough to take women from the physical toil of housekeeping and put them on equal wheeling with male commuters? But for the normal (or not so normal these days!) healthy body, let taste, not diet, remain the final arbiter. No doubt those in bondage to the golden car will regard this as mere folly and scandal, yet that symbol of supposed "freedom" increasingly becomes the premature reaper, whether we are saturated or unsaturated.

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Isolative sound-change

Sir,—I should like to propose the following explanation for the isolative vowel sound-change from Anglo-Saxon *ā* through Middle English ǭ to Modern English *ō* (as in *mote*) instanced by Alan Ross (April 22, page 664).

If you open your mouth, say "ah", and continue the utterance while gradually allowing your mouth to relax and close you will find yourself recapitulating, in the course of seconds, the same sequence of sound changes as your correspondent cites as having occurred over centuries, and for the same basic reason, namely a gradual tendency to open the mouth less wide to make the vowel sound—in a word, laziness (It is interesting to compare the current marked tendency for the modern Persian *ā*—which is nearer to "aw" than to "ah"—to lapse into ū, for example, *Teherān* to *Teherūn*).

In some areas of England, where vowels are stronger, the transition has not yet reached the third stage. To give a ready example, listeners to the BBC television series "When the Boat Comes in" may have noticed that the pronunciation there of *boat* still approximates to the Middle English *bǭt*—as its surviving spelling indeed suggests it should.

I believe that the laziness principle also explains the tendency to lapse from *fire* to *far*, and so on. And surely it accounts for the tendency for our short vowels—*a* as in *Indian*, *e* as in *absent*, *i* along with *o* as in *station*, and *o* as in *onion*—to lapse into the basic *un*-sound found onomatopoeically in *grunt* But I would not suggest that it could account for all isolative vowel changes, because undoubtedly there is a complex of physical and psychological factors involved.

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news and views

CO$_2$ variations and the Southern Oscillation

from John Gribbin

THE so-called "quasi-biennial" oscillations of the atmospheric and oceanic system, which produce variations in weather parameters with 'periods' of anything from a year to at least half-a-dozen years, but usually averaging between two and three years, are among the best known and most intensively studied climatic phenomena—but not necessarily the best understood. One very important member of this family is the Southern Oscillation, which is most clearly identified by variations in pressure with a timescale of two to two and a half years over the Southern Hemisphere; these variations are often very highly correlated between the two key areas around Jakarta (Indonesian low) and Easter Island (South Pacific high), and to a lesser extent the variations can be correlated with features of the Northern Hemisphere circulation. The Southern Oscillation is not, then, some local or even hemispheric phenomenon, but rather a particularly obvious manifestation of the general tendency of the atmosphere/ocean system to vary on this kind of timescale, and study of just how this oscillation does interact with other parts of the atmosphere/ocean system is important in the context of understanding quasi-biennial oscillations in general—and, ultimately, producing better forecasts.

Lamb has succinctly summed up the situation (*Climate· Present, Past and Future,* **1,** Methuen, 1972): "the Southern Oscillation and its roughly 2- to 3-year periodicity can clearly be taken to involve an alternation in the pattern and rate of heat distribution affecting much of the world and its wind circulation . . . the Southern Oscillation should be seen as related to the liability of the equatorial Pacific to some of the biggest and most extensive anomalies of sea surface temperature in the world". These anomalies have one very obvious and significant effect in the phenomenon known as El Nino, which brings high rainfall to generally arid regions of the equatorial Pacific, and although the link between this phenomenon and the Southern Oscillation is not entirely clear, anomalous conditions at the Easter Island high do seem to be involved.

It is with the importance of an understanding of the Southern Oscillation to our understanding of the whole atmosphere/ocean system in mind that the contribution by Bacastow on page 116 of this issue of *Nature* should be read. Bacastow claims that after a great deal of filtering to remove both seasonal effects and the long-term increase attributed to man's activities the record of carbon dioxide variations in the atmosphere indicates a correlation with the Southern Oscillation. Critics might take Bacastow to task for making the most of somewhat limited statistical evidence which he admits involves adjustments in some cases so that "the fit appeared reasonable to the eye", and which even then end up with correlations which although high enough to be interesting are not conclusive on the basis of these limited data alone. In addition, the very small variation in carbon dioxide levels (less than one third of 1%) seems to occur before the changes in the Southern Oscillation which might be expected to drive them. Nevertheless, although it seems unreasonable to suggest that the carbon dioxide variations might drive the oscillation, there remains the important possibility that both are being driven, or at least modulated, by some outside influence which has yet to be identified.

The somewhat tentative nature of the model proposed is, however, indicative of the tentative state of our understanding of the way the atmosphere and ocean interact. It is easier to appreciate that a thorough understanding of atmospheric variations can only come hand-in-hand with a thorough understanding of oceanic variations, but much harder to see where to begin to tackle the enormous problems involved in developing such an understanding. If the views of some climatologists are to be taken at face value, there is now also an element of a race against time to determine the natural patterns of such change before they are destroyed by man's activities—the controversy about carbon dioxide levels and man being a case in point—and prediction of these anthropogenic effects can only be achieved after an understanding of the natural processes has been developed. The specific case of the interaction between the Southern Oscillation and carbon dioxide uptake of the southern ocean is of direct relevance to the controversy over carbon dioxide pollution and its effects on the atmosphere and oceans; the broader view—what drives all these quasi-biennial oscillations—is perhaps of even greater importance now that the world food production and distribution systems can be so easily shaken by "bad" weather, as we have seen in recent years. ☐

More speculation on human evolution

from Robin Weiss

WHILE fossil finds hit the headlines, comparative biochemistry also has its place in the discourse on the origins of our species Two papers in this issue of *Nature* take up this discussion, one from the viewpoint of amino-acid sequences in proteins, the other of nucleotide sequences in DNA Romero-Herrera *et al.* (page 162) discuss the amino acids residing at two positions in the highly conservative muscle protein, myoglobin, which accepts oxygen from the blood. On the basis of the amino-acid residues in positions 23 and 110 of the myoglobin chain, these authors draw up 'cladograms' or evolutionary trees that might indicate the relationship of man to the higher apes The most economical cladogram in the sense of providing the minimum number of point mutations in the gene for myoglobin shows the orang-utan diverging from the other higher apes and man before the gibbon does. The problem with 'economy' of mutation is that it assumes that mutations are always selectively disadvantageous. This may be true for myoglobin, but residues in several proteins must be analysed before one cladogram can emerge as the clear choice.

Benveniste and Todaro's paper, also in this issue of *Nature* (page 101), concludes that during most of the Pliocene era, man's direct ancestors lived in Asia, or at least not in Africa. Therefore they would argue that the recent fossil finds by Richard Leakey in Kenya and by Johanson and Taieb in Ethiopia (announced at a news conference two months ago, but not yet published) that appear to push African records of *Homo* back into the Pliocene may not be the ancestors of our present day species.

Benveniste and Todaro's conclusions arise from ingenious arguments based on tenuous evidence. They have used the technique of DNA hybridisation by which the melted strands of DNA will reanneal with complementary sequences. When melted DNA from two species is allowed to reanneal only complementary nucleotide sequences will do so, and the degree of mismatching within those sequences can be measured as the depression in melting temperature in the 'hybrid' DNA compared with that of truly homologous DNA Thus the degree of genetic relatedness of species is determined by the extent of DNA hybridisation, and by the temperature at which 50% of the hybrid becomes dissociated. Benveniste and Todaro's annealing studies of DNA prepared from tissues of humans and apes broadly confirm earlier work, notably of Kohne and his associates at the Carnegie Institute of Washington, that the non-repetitive DNA sequences of man are more closely related to those of the African great apes, the chimpanzee and gorilla, than those of Asian apes. So far so good, in that these sophisticated biophysical studies of genetic material are consistent with, though perhaps less discriminating than, the morphological studies of classical primatologists or the eyes of children visiting the zoo.

Benveniste and Todaro then proceeded, however, to analyse a tiny fraction of DNA representing about 10^{-7} of the non-repeating sequences, and possessing the coding capacity for a small protein at the most This set of sequences is distantly related to part of a C-type virus endogenous to baboons, that is, a virus whose genes are incorporated into the host DNA and which is therefore inherited as a Mendelian trait. By synthesising a radioactive DNA 'probe' complementary to the genes of the activated baboon virus one can screen for related 'viral' sequences by annealing the probe to DNA prepared from normal tissues of other species of primates, including man. About 9% of the probe anneals to chimpanzee and gorilla DNA but only 3% to human DNA and to that of Asian species of ape. The extent of annealing is so small and the mismatching so great, that the melting temperature of the hybrid DNA can only be estimated by a crude extrapolation. Benveniste and Todaro do not think that man is more closely related to Asian apes than African ones since the overall cellular DNA hybridisation patterns indicate the converse. Their thesis is that African apes have conserved a larger fragment of DNA related to the baboon virus than have either Asian apes or man; that this conservation of viral DNA sequences results from natural selection due to some factor in the African as opposed to the Asian environment; that this factor is the baboon virus itself (and that of ancestral baboons) transmitted as an infectious agent; and that the conserved DNA sequences somehow confer immunity to infection of the host by the baboon virus. If man was not exposed to the baboon virus during the Pliocene era, there would be no selection to conserve these DNA sequences and they would diverge as fast as 'viral' sequences have done in other non-African Old World primates.

To launch such a complicated hypothesis on the difference between 9% and 3% of grossly mismatched homology of DNA sequences seems far-fetched. But Benveniste and Todaro show that it fits a general pattern in that sequences in Old World monkeys that are more closely related to the baboon virus have also diverged at a much higher rate among Asian species than among African species. Moreover the evolutionary arguments do not seem to me implausible, indeed they are familiar. There is some evidence in other species (chickens, for example) that endogenous viral gene expression can confer resistance to re-infection and there is also striking evidence from Benveniste and Todaro's previous studies (*Nature*, **252**, 456; 1974) that a C-type virus related to the baboon virus has been transmitted to the genome of the ancestor of the domestic cat (of North African origin) but not to an Asian species of the same genus.

Entertaining as these arguments are, on the basis of the evidence presented here they might have been more wisely restricted to the laboratory coffee room. The argument for man's Asian origin is based on the weak homology of a small fraction of a viral probe. There are no data to determine whether the conserved sequences in different African primates represent the same portion of the probe and indeed the viral sequences 'conserved' by natural selection actually diverge more rapidly than the non-repetitive host DNA as a whole.

An implicit corollary of Benveniste and Todaro's hypothesis is that man is susceptible to horizontal infection with C-type viruses of the baboon type, and that these viruses could be pathogenic to man. C-type viruses closely related to baboon viruses have been isolated from human leukaemic cells by Gallo and his associates and from fibroblastic cells by Panem and Kirsten. I have argued previously that zoonosis, ancient or modern, may be an important concept in the transmission of pathogenic C-type viruses, and that we should not be too perturbed to find that 'human' isolates resemble animal viruses Benveniste and Todaro's paper hints that they may now be coming to the same opinion. □

A hundred years ago

At the Crystal Palace Aquarium the hatching of the spawn of Axolotls has been successfully accomplished. There are three young "broods," and some still unhatched spawn, so that the changes in growth during the first few weeks can be seen all together.

A PLAGUE of Field Voles (*Arvoricola agrestis*) has recently visited some of the pastoral farms of Upper Teviotdale and the adjoining districts, which has led to the appointment of a committee of the Farmers' Club of the Locality for the purpose of estimating the amount of damage done. This was found to be very considerable, the vermin eating the pale and succulent bases of the grass, as well as the young shoots. No great destruction of other vermin has occurred in the district, so it cannot be said that the Voles have become particularly abundant from that cause. Similar plagues have before now occurred in the New Forest.

THE *Geographical Magazine* for May contains an article on the prospects for the Arctic Campaign of 1876. While we must be prepared for the necessity of the ships spending a second winter in the north, the writer thinks that possibly the *Pandora*, which goes out this month for news and letters, may meet them coming out of Smith Sound, "with their work done, their great enterprise completed." There is also an article, with map, on the Island of Socotra, which the British Government have arranged to occupy, and another by M. Venyukof on New Maps of Mongolia.

from *Nature*, **14**, 35, 36, May 11; 1876.

Sociobiology: a reply

WE are sad and disturbed at the tone of the article by Robert May (*Nature*, **260**, 390; 1976) on E. O. Wilson's *Sociobiology: The New Synthesis*. A widespread debate, originating in America, is taking place around this book and its adoption as a text for college courses. May's article attempts to sidestep the issues being raised by this debate and to remove the discussion to the rather more traditional and secluded arena of scholarly exchange. The term 'debate' in this context is, we recognise, distasteful to many scientists with a traditional view of science and we use it advisedly. We feel, however, that the effects of science upon everyday thought and upon political doctrines are issues that cannot be resolved by courteous sword-crossing within the language of 'pure' science itself.

On the contrary, the issues raised evoke the deepest feelings about power and its abuse, and about social injustice in ways that inevitably force a confrontation between individuals and groups holding disparate sets of values. We must learn to see what May describes as 'a certain incivility' as a function of the importance of such debates/confrontations. Since consideration of the social bases and the social effects of science should be recognised as being integral to science and its practice, such impassioned and political arguments should appear more regularly in the columns of *Nature* and *Science*

More importantly, May's focus on the so-called incivilities deflects attention from the serious nature of the issues posed by the publication of Wilson's book. The group of American critics (the Sociobiology Study Group of Boston Science for the People) to whom he refers, see in Wilson, essentially correctly we believe, a prime example of the scientific specialist (the naturalist in the broad classical sense) whose prolonged study of the bee and the ant have fostered in him a rather conservative expectation of the human capacity and potential for change. While such conservatism may well be largely unconscious and not overtly political in expression, untold damage is wreaked on 'informed' people's capacity to think critically about social institutions. By attempting to fit *Homo sapiens* into his evolutionary biological schema Wilson jeopardises any attempt to change present patterns of social inequality. As such it seems to us that 'a certain incivility' is more than reasonable.

Wilson's American critics have raised important objections to his work. They show how his case is based on speculation. He postulates genes, or groups of genes, for such traits as spite, military discipline, blind faith, and indoctrination. He uses metaphors from human societies to describe animal societies and in so doing imputes barter, religion and magic to animals. He closes off the theory from tests against the real world by postulating, for example, multiplier and threshold effects which make every observation fit the theory. May attempts to belittle this critique. "To suggest, for example, that the evolutionary considerations which determine the mating systems of mammals and birds have any light to shed on the tensions and asymmetries observed in human sexual relationships is [according to May] to invite reflexive dismissal as a 'sexist'"! Tensions and asymmetries are delicate words indeed to express centuries of economic and social exploitation of women. And in fact, Wilson does try to establish the inevitability of sexism by asserting it has a genetic basis. In an article in the *New York Times* (October 12, 1975) he states concerning the division of labour between the sexes: "This strong bias persists in most agricultural and industrial societies and on that ground alone appears to have a genetic origin . . My own guess is that the genetic basis is intense enough to cause a substantial division of labour even in the most free and most egalitarian of future societies . . . ."

That our society should continue to be so susceptible to such arguments expresses both a past and present deference to expertise in general and a deference of social science to biology in particular. At present we learn in school level biology courses and thereafter the roster of emergent properties that distinguishes life from inorganic matter. Life transcends the purely physical and chemical laws that govern inorganic matter, although matter in motion remains its physical basis. We must ask why, in our society, the human sciences cannot emancipate themselves from biology in the same way that biology is emancipated from the physical sciences We are unique animals who, while having a physical basis in organic life, transcend it through language and culture.

The final issue is, of course, how does the cultural *status quo* change? It seems obvious to us that social change occurs when humans set about self-consciously to change unacceptable oppressive and outmoded social conditions.

*The Science as Ideology Study Group of the British Society for Social Responsibility in Science**
19 Hartham Road, N7.

*Members include: Anne Cooke, Jonathan Cooke, Mary Fuller, Dorothy Griffiths, Susie Orbach, Joe Schwartz, Bob Young.

ROBERT MAY REPLIES: The above letter makes plain the differences of opinion between its authors and myself as to the place of passion and politics in science In particular it is true that I represented the controversy over the political significance of *Sociobiology* as only a small (and unfortunate) corner of a large canvas; I think that is the balanced view.

It should be emphasised that, unlike other recent controversies, the dispute over the political implications of *Sociobiology* is not a clash over substantial and well-defined issues. The Science For The People people do not attack Wilson's book but rather their own chimaerical version of it; in Wilson's words they "furnish me with a political attitude I do not have and the book with a general conclusion that is not there". Readers in any doubt on this score should see the recent exchange between the US Science for the People Study Group and Wilson in *BioScience* (**26**, 182–190; 1976) and the impartial news article in *Science* (**191**, 1151–1155; 1976). □

Panacea for injured nerves

from a Correspondent

WHEN a nerve innervating a muscle is cut the effect on the postsynaptic cell is profound The muscle atrophies, as shown by a loss of mass and of proteins, there is a marked reduction in the cholinesterase activity present in the tissue, and there is a considerable increase in the number of extrajunctional receptor molecules. Since the onset of these effects is related to the length of the nerve stump left associated with the tissue, and since they can be duplicated by blocking release from the nerve terminal, it has been proposed that nerves exert trophic influences on the tissues they innervate (Guth, *Physiol. Rev.*, **48**, 645; 1968). Considerably less attention has been focused on a trophic effect in the opposite direction, from tissue to nerve, although this possibility was first implied 18 years ago (Brown, *J. Physiol., Lond.*, **142**, 7P; 1958). This situation is now being rectified.

Two approaches have been used to study this suggested transfer of information from the post to the presynaptic cells. First, morphological techniques have been used to examine the effects of damaging the postsynaptic cell on presynaptic or synaptic ultrastructure and, second, biochemical correlates to these structural changes have been sought.

The morphological data have now provided unequivocal evidence that damage to a sympathetic ganglion cell, by cutting or ligating its axons, leads to a retraction of the innervating nerve and the insertion of a Schwann cell process between the pre and postsynaptic elements (Matthews and Nelson, *J. Physiol., Lond*, **245**, 91; 1975; Purves, *J Physiol., Lond.*, **252**, 429; 1975). The synapse thus becomes ineffective in that there is no transmission through the ganglion. The reversibility of the process depends upon the method of damaging the axons. If the possibility of regeneration and reformation of functional postganglionic synapses is excluded, then functional synapses on the ganglion cells do not reform either, and many cells die (Purves, *op. cit*). The preganglionic nerves appear ultrastructurally normal although their size may have changed (Matthews and Nelson, *op. cit*). Similar effects on the innervation of motor neurones were found previously (for example, Blinzinger and Kreutzberg, *Z. Zellforsch.*, **85**, 145; 1968; Cull, *Expl Brain Res.*, **20**, 307; 1974).

If biochemical rather than morphological changes are studied, however, it becomes apparent that the innervating nerves have been altered. For example, Jensen-Holm and Juul (*Acta pharmac. toxicol.*, **28**, 583; 1970), have shown that damage of ganglion cells by guanethidine results in a loss of cholinesterase activity from both the pre and the postsynaptic elements. Black, Hendry and Iversen (*J. Physiol. Lond.*, **221**, 149; 1972) found that destruction of the ganglion cells, using 6-hydroxydopamine or anti-nerve growth factor, prevents the normal development of choline acetyltransferase, the one enzyme known to be unique to the incoming cholinergic nerves and their terminals. In each of these cases it could be argued that the loss of synaptic contact with the ganglion cells removed the source of the information which the innervating nerve required to maintain its biochemical integrity. Similar changes have also been found in motor neurones. In these experiments the effector (muscle) cell has been damaged and the biochemical response to the injury measured in the innervating nerve, but not in those which impinge upon it. It is intriguing that the response of the nerve depends upon the type of damage inflicted on the muscle. For example, atrophy induced by inactivation of the acetylcholine receptors (using an α-neurotoxin) results in a decrease of choline acetyltransferase activity in the nerve terminals and axons. In contrast, no change in the levels of the enzyme was found when muscle atrophy was induced by treatment with botulinum toxin, a poison whose exclusive action is presumed to be a block of release from the presynaptic nerve terminal (Giacobini-Robecchi, Giacobini, Filogamo and Changeux, *Brain Res.*, **83**, 107; 1975). Tenotomy-induced atrophy also results in a decreased level of choline acetyltransferase activity in nerve endings whereas that provoked by cortisone treatment does not (Diamond, Franklin and Milfoy, *J. Physiol., Lond.*, **236**, 247; 1974). Hypertrophy which resulted from hyperactivity of the muscle did not result in increased levels of the choline acetyltransferase. Thus, it is not whether you injure a muscle or not that is important, or whether you alter its mass, it is the mechanism you use to produce the functional disturbance that counts.

There are also reasons for suggesting that the neurones which synapse on the motor nerves might be altered when these nerves are damaged. Giacobini-Robecchi *et al.* (*op. cit.*) have shown that when the α-toxin, but not the botulinum toxin, is used, there is a significant decrease in the size of the sciatic nerve, its spinal roots and of the corresponding sensory ganglia.

All these results lead to one conclusion. If neurones or innervated cells are damaged in certain ways then this will ultimately modify the neurones which make synaptic contact with them. The question which currently excites a great deal of interest is how, and in what form, is the information transferred from the postsynaptic cell to the presynaptic nerve terminal and even across several synapses and well into the central nervous system?

Only one molecule, nerve growth factor, has so far been implicated in the transfer of this information. Nerve growth factor is a protein which, as its name suggests, is able to stimulate the growth of neurones but its action is limited to those of the noradrenergic and sensory nervous systems. It is also a molecule which is taken up, fairly selectively, by the nerve terminals and, possibly, the cell bodies of the neurones present in these two systems. As well, nerve growth factor is able to prevent some of the biochemical changes which result from postganglionic axotomy of noradrenergic cells (Hendry, *Brain Res.*, **94**, 87; 1975). Interestingly, this protein can also prevent the depression of ganglionic transmission which results from crushing the postganglionic nerves, if it is supplied to the ganglion at the time of nerve injury (Purves and Njå, *Nature*, **260**, 535; 1976). Thus an additional role of nerve growth factor could normally be to maintain the synaptic contacts on a cell and they, in their turn, could then help to maintain certain biochemical parameters of the postsynaptic cells. It is likely that this effect of nerve growth factor is achieved by its transfer from the nerve terminals to the cell bodies. Since there is no maintenance of synaptic junctions, nor protection from axotomy, unless exogenous nerve growth factor is administered at the time of injury, it is unlikely that normal circulating levels of the protein are sufficient to influence the cell bodies directly. It needs to be concentrated first in the nerve terminals and then carried to the cell body.

Nerve growth factor cannot, however, be a universal panacea to injured nerves and their processes Motor nerves, which respond to injury much as sympathetic nerves do, seem unable to concentrate and transport nerve growth factor (Stöckel and Thoenen, *Brain Res.*, **85**, 337; 1975). A direct sensory link transmitting this specialised information from the injured muscle to the nerve is also unlikely (Giacobini, Filogamo, Weber, Boquet and Changeux, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1708; 1973) so it is probable that the signal can be transferred directly. The nature of the signals and how they are transferred, are not known, but the identification of these signals must number amongst the most fundamental questions that neuroscientists could wish to answer. ☐

Radon to predict earthquakes

from Peter J. Smith

AMONG the many potential earthquake predictors investigated in recent years, one of the most ignored, at least in the West, is the apparently premonitory change in the emission of radon. In a brief conference abstract King (*Eos*, **56**, 1019; 1975) recently reported that monitoring of the radon concentration in soil gas was begun in May 1975 at 18 sites along an active 60-km segment of the San Andreas fault in central California and that the first results indicated "spatial and temporal variations which appear to correlate with local seismicity". At the same meeting Teng *et al.* (*Eos*, **56**, 1019; 1975) also reported that a sampling network had been established along the San Andreas fault from Cajon to Gorman in order to measure systematically the radon content of groundwater. By December 1975, however, no definitive results had emerged, although there were "some interesting variations when compared to the local earthquakes".

The latter study in particular was inspired by a long series of groundwater radon measurements made in Russia; and it would seem that until now Russia and China have been the only two countries to pursue assiduously the possibility of predicting earthquakes by radon emission variation. Following a visit to China in 1974, for example, Press *et al.* (*Eos*, **56**, 838; 1975) concluded that "some of the work in China on radon emanation as a precursory effect is to our knowledge as good as that being done anywhere in the world". Apparently, the waters in at least several tens of wells are being monitored continually (sampling normally once or twice a day) using electrometers which measure the radon-emitted α-particle fluxes.

The result of this activity is that a precursory increase (or, more rarely, a decrease) in the radon content of water has been detected for at least six Chinese earthquakes ranging in magnitude from 4.3 to 7.9. The precursor times ranged from a few days to a few tens of days, and in most cases the radon anomalies were observed within 110 km of the subsequent shock. In the case of the magnitude 4.9 earthquake of June 6, 1974, for example, the radon anomaly, observed at a distance of 49 km, appeared about 15 days before the shock and produced a radon flux 2–3 times that of the normal background.

In view of this success it seems likely that experimental interest in the radon precursor will increase rapidly in the United States and elsewhere in the near future. In the meantime, however, Pierce (*Geophys. Res. Lett.*, **3**, 185; 1976) has pointed to a consequence of variations in radon emission which could well extend observational methods beyond the direct measurement of radon itself. His premise is that an increase in atmospheric radon resulting from the release of radon from the ground should have an effect on atmospheric electricity—in particular on the atmospheric electric field and on the conductivity of the atmosphere. As long as the effect is large enough, therefore, the monitoring of such quantities should reveal the radon precursor indirectly.

To estimate just how large an effect could be expected, Pierce has set up a highly simplified theoretical model of atmospheric electricity which is meant to be "illustrative rather than definitive". The rationale behind this rough approach is that both the physics of the situation and the numerical values involved are so uncertain that a more sophisticated model would hardly be justified. Moreover, in related work, simplified models have turned out to be surprisingly effective. Be that as it may, the model predicts that a precursory increase (of 100%) in radon emission would lead to an increase in fair weather conductivity of about 50% and an average decrease in electric field of about 30% (20% by day, 40% at night). On this basis, the obvious parameter to measure is conductivity. Unfortunately, however, it is technically much more difficult to measure conductivity than electric field. The field change may be smaller but is easier to record, and 30% might just be detectable.

But only under ideal conditions. A field anomaly of 20–40% represents an absolute change of 20–150 V m^{-1}, and clouds and precipitation can produce changes of several hundred volts per metre, without a hint of radon variation, by generating space charge. Moreover, even in fair weather conditions, such agencies as waterfalls, surf, dust, power lines, aerosols and air pollutants may create space charge and hence perturb the normal field. With problems like these, ideal conditions might be considered unattainable. Nevertheless, Pierce believes that they may be nearly achieved in the rural areas of some Californian valleys in the summer. Accordingly he recommends the addition of electric field monitoring equipment to experiments such as those being carried out by King.

The difficulties with environmental conditions suggest that electric field anomalies are unlikely to be reliable earthquake predictors by themselves, although they could provide valuable support to other observed precursors. It is perhaps worth pointing out, however, that atmospheric electric field monitoring has actually been used in at least one earthquake study. Kondo (*Mem Kakioka Magn Observ.*, **13**, 11; 1968) recorded occasional field decreases of as much as 20% during the Matsushiro (Japan) earthquake swarm of late 1966. He even suggested that the changes might be related to an enhanced radon content of the atmosphere, but never pursued the idea in detail. □

Ionising helium

from Peter Knight

THE ionisation of an unperturbed helium atom, starting from its ground state, would require an energy equivalent to that of 22 photons from a 1.06 μm laser. The corresponding "22-photon ionisation" rate would depend upon the 22nd power of the intensity at modest laser intensities, provided that there are no laser temporal coherence effects (due to multimode operation for example) and provided one is far enough away from any possible atomic resonances which would modify the non-linear intensity dependence of the absorption. The fact that 22 photons from a 1.06 μm laser seem to be needed to ionise a helium atom when the laser intensity exceeds 10^{15} W cm^{-2} is, however, a surprise. A group from Saclay has recently reported results on the multiphoton ionisation of rare gases by 30 ps laser pulses at 1.06 μm (Lompre *et al.*, *Phys. Rev Lett.*, **36**, 949–952; 1976) which seem to show just that.

Their laser system produces linearly polarised radiation with a linewidth of 0.8 Å centred upon 10 643.5 Å—chosen to avoid atomic resonances. It is operated to generate single pulses of duration 30 ps so that very high peak focused intensities (typically in excess of 10^{15} W cm^{-2}) may be generated, and so that coherence effects may be avoided. The variation of the number of photoions produced as a function of laser intensity at the focal region is investigated. Then for a single pulse away from an atomic resonance one would expect from the perturbation theory of K-photon multiphoton ionisation a transition rate proportional to the Kth power of the intensity. The following table, taken from Lompre *et al.'s* paper, compares K_0, the next integer greater than the atomic ionisation energy divided by the laser photon energy, with K_{exp}, the slope of the log-plot of the number of photoions produced as a function of intensity for five rare gases.

Atom	K_0	K_{exp}
Xe	11	11±0.5
Kr	13	13±0.5
Ar	14	14±0.5
Ne	19	20±2
He	22	23±2

K_0 is essentially the minimum number of photons at the laser frequency needed to ionise an unperturbed atom.

So even at the high intensities used by the Saclay group (7×10^{14} W cm^{-2} to 1.1×10^{15} W cm^{-2}), K_0 and K_{exp} are in good agreement. This is surprising since for these field intensities, the atom certainly is not unperturbed: the peak laser electric field E approaches or even exceeds the internal atomic electric field E_0 responsible for the atomic binding. In such circumstances it is not obvious why the ionisation energy of the unperturbed atom is such a crucial factor. Theoretical work by Keldysh (English translation: *Sov. Phys. JETP*, **20**, 1307; 1965) had suggested that electron tunnelling ionisation rather than multiphoton ionisation would be dominant for $E>E_0$. The Saclay group, approaching $E>E_0$ for the first time in a multiphoton ionisation experiment see no evidence of this yet. If tunnelling occurs present theories predict an intensity-dependent K_{exp}, but this has not been observed. Clearly further experimental work is needed, but already the Saclay group's results indicate a need for new theoretical approaches to this difficult problem. In particular, the theory of tunnelling urgently needs to be re-examined. □

Junctions and synapses in development

by Eleanor Lawrence and Miranda Robertson

A meeting on Intercellular Junctions and Synapses in Development was held in Cambridge on April 6–8, 1976, under the auspices of the International Society of Developmental Biologists and University College London. The meeting was organised by Joan Feldman of University College and the proceedings will be published by Chapman and Hall.

SYNAPTOGENESIS and the appearance of other junctions during development raise such different questions that they tend to be considered separately. But answering those questions may involve much the same technology, and it is at the level of technique that the cross-fertilisation encouraged by combining them in the same symposium may prove fruitful.

Gap junctions, the main topic of the meeting, are structures still in search of a function. The structural and permeability properties of the gap junction strongly implicate it as the communication channel for the passage of ions and small molecules between cells, but—except in the myocardium—there is no direct evidence as yet for any physiological function *in vivo.*

By freeze-fracture electron microscopy gap junctions appear as patches of intramembrane particles arranged in hexagonal arrays. The model that was proposed originally for the gap-junction structure by N. B. Gilula (Rockefeller University) was of a dumb-bell shaped protein complex spanning the two apposed membranes and penetrated by a central hydrophilic pore. This model is supported by analysis of X-ray diffraction patterns of isolated gap junctions which has also revealed a six-fold symmetry around the central pore. (D. Goodenough, Harvard Medical School). C. Perrachia (University of Rochester, New York) also proposes a structure of six dumb-bell shaped subunits as an explanation of the different shapes the isolated gap-junctional particles take in the electron microscope.

Electrophoretic separation of isolated gap junction proteins suggests that there are two main subunits but their true molecular weights are far from clear mainly due to proteolysis during the isolation procedure. Attempts by Goodenough and Don Caspar (Brandeis University) to provide a definitive structure for the gap-junctional particle by the low dose electron microscopy technique used so successfully on bacteriorhodopsin, have unfortunately run into difficulties, since the hexagonal arrays of particles turn out to be not sufficiently regular to give any clear results so far.

A possible structural change which can be correlated with functional uncoupling is the apparent contraction of the gap junctional lattice on fixation, when the particles become more closely packed and smaller in diameter (found independently by Goodenough and Perrachia). Goodenough suggested that that might close the channel by blocking the central pore.

Ionic (electrical) and metabolic coupling in cell culture have been clearly correlated with the presence of gap junctions. Correction of metabolic defects in mutant cells in contact with normal cells, and other biochemical methods, have been used to demonstrate that a wide variety of small cellular molecules such as amino acids, sugars and nucleotides can cross the gap junction: cellular macromolecules do not cross. (John Pitts, University of Glasgow) Birgit Rose (University of Miami) has found a 1,000 molecular weight upper limit for synthetic polypeptides. So far she has found no similar upper limit for synthetic oligosaccharides which are apparently still going through at molecular weights of 1,300. In *Chironomus* at least, the permeability of the gap junction can be regulated by Ca^{2+}, which at sufficiently high concentrations at the junction blocks the passage of an electrical current (Rose).

Although the idea that gap junctions have an important role as a communication channel between like cells during development is attractive it is thus far unsupported by any convincing evidence. J.-P. Revel (Caltech), who claimed to have found his own results almost impossible to interpret, remarked pessimistically that the existence of a fine telephone system doesn't necessarily mean that there's anybody talking down it, or even if there is, that they're talking sense He finds that during neurulation in the chick embryo, not only do the gap junctions present in the epiblast before invagination of the neural groove disappear as the neural groove develops but the tight junctions in that region also become disrupted and all that can be seen when the neural tube closes are scattered groups of a few particles where the tight junctions should be. It is impossible to tell whether those are tight junctions disappearing or gap junctions forming or whether the gap and tight junctions are transmutable.

Anne Warner (Royal Free Hospital, London) has elected to follow the changing patterns of electrical coupling in developing axolotl and *Xenopus* embryos. She presented a beautifully clear demonstration of electrical coupling changes during somite formation in the axolotl and *Xenopus.* In both embryos, the unsegmented mesoderm cells are strongly coupled to each other. Prospective somite cells become uncoupled before or during somite formation and in *Xenopus* the cells in the newly-formed somite recouple not only to each other but to adjacent somites as well. In the axolotl the formed somites do not couple to each other. Gap junctions between coupled cells have been demonstrated in this system (Blackshaw and Warner, *J. Physiol., Lond.*, **255**, 209–230; 1976).

S. W. de Laat (Hubrecht Laboratory) directly challenged the assumption that ionic coupling seen between the cells of the first cleavage to fifth cleavage *Xenopus* embryo is mediated by gap junctions, by suggesting that the electrical coupling is due to the generally lower permeability of the intercellular membranes relative to the outer bounding membranes rather than to the presence of specialised low resistance junctions (gap junctions) in the cleavage membrane.

But during discussion it was pointed out that although these intercellular membranes are relatively particle-free, even the few particles that can be detected could account for the ionic coupling if they were acting as individual communication channels.

R. S. Decker (University of Dallas) has developed a simple and elegant system for studying the formation of gap junctions during differentiation. He has followed the progress of junction formation during hormone-induced differentiation *in vivo* (hypophysectomised *Xenopus* embryos responding to thyroxin) and *in vitro* (adrenal cortical tumour cells responding to ACTH) and has demonstrated that both RNA and protein synthesis are required.

The existence of defined junctional mutants could aid the analysis of the functional significance of the gap junction considerably. Pitts reported that no single-step mutations have yet been found. W. Loewenstein (University of Miami) has been studying the behaviour of somatic cell hybrids between communication competent normal human cells (which possess gap junctions) and communication incompetent mouse cells (lacking junctions) and finds that the early heterokaryons resemble the normal parent. But as the human chromosomes are gradually lost over the generations the cells revert to communication incompetence. Loewenstein has been unable to ascribe reversion to the loss of any particular human chromosome or chromosomes but believes that not more than two are involved.

The principal problem in synaptogenesis is not its *raison d'être* but its mechanism—how do innervating fibres recognise their target cells, how specific is that recognition and what part is played by competitive interactions between innervating fibres? C. Sotelo (INSERM) reviewed morphological evidence from mouse cerebellar mutants that in the absence of the normal postsynaptic sites, growing nerve fibres will make alternative synapses and conversely that in the absence of their normal innervation postsynaptic structures can attract nerve fibres which would not normally reach them

The existence of relatively nonspecific specialised sites intrinsic to the membrane of postsynaptic cells in the CNS was emphasised by G. Raisman (MRC Mill Hill) who described the reinnervation of denervated adult rat superior cervical ganglion cells by the vagus and the hypoglossal nerves at the sites of the postsynaptic thickenings originally occupied by preganglionic nerve fibres.

The molecular nature of the recognition system for postsynaptic membrane is unknown, although one obvious possibility is the neurotransmitter receptors themselves. Most attempts to test that possibility in the central nervous system have failed. But J. Freeman (Vanderbilt University) has succeeded in showing that the retinotectal system in *Bufo* is cholinergic, and claims that by applying the cholinergic receptor blocker α-bungarotoxin to small areas of the developing optic tectum he can create localised defects in the electrophysiological map of the visuo-tectal projection.

Detailed studies on the behaviour of receptors are much easier at the neuromuscular junction, where it is believed that the increased density of receptors at the postsynaptic membrane is a consequence of innervation rather than its cause. G. Fischbach (Harvard University) however presented evidence that chick skeletal muscle cells *in vitro* develop areas of high receptor concentration in the absence of innervation. These receptor "hot spots" are detected by intracellular recordings from the muscle as areas of heightened sensitivity to focal stimulation with ACh, which confirms Nirenberg's report that local concentrations of α-bungarotoxin receptor binding sites exist on muscle cells *in vitro*.

The stability of concentrations of receptor sites *in vivo* was emphasised by C. Slater (Newcastle General Hospital), who finds that the peaks of sensitivity that develop in denervated adult rat soleus on reinnervation with the superficial fibular nerve persist for at least a week after section of the fibular nerve.

The question of competitive interactions in the specification of neuromuscular junctions formed in developing mammals was addressed by D. van Essen (University College London), who reported that the multiple innervation found in neonatal soleus is eliminated by 2–3 weeks after birth, through the withdrawal of supernumerary terminals by the motor axons. This shrinkage occurs however even when half the competing terminals are removed by the early section of one of the ventral roots, and some muscle fibres are left vacant. In experiments in which an immature muscle was denervated and reinnervated by a foreign nerve which was then forced to compete for synaptic sites with the regenerating terminals of the original nerve, multiple innervation persisted only where the synapses were at least 1 mm apart.

The resolution of problems in synaptogenesis seems likely to be found in the properties of the membranes; the properties of gap junctions will remain a series of tantalising hints until someone can define a disruptive effect of blocking them *in vivo*. □

Radiological data collection

from a Correspondent

> A meeting on Radiological Protection Data: Collection, Recording and Use, organised by the Society for Radiological Protection was held in London on April 6, 1976.

A NATIONAL register of radiation workers is to be set up in this country, S. Rae, Assistant Director (Medical) of the National Radiological Protection Board, told the meeting. Negotiations for establishing the register, initially to cover workers in the nuclear energy industry, are now being conducted. It is hoped to extend it to include for example, industrial radiographers and miners exposed to radiation.

The purpose of setting up such a national register will be to carry out an epidemiological study of cancer mortality in radiation workers and to look for effects of occupational radiation exposure on the natural rate of cancer induction. The register will follow, for at least 25 years, the radiation exposure of a large population of radiation workers and will match this with information obtained from the records of the Office of Population Censuses and Surveys about the age at death and the cause of death of these workers.

He indicated that calculations, based on current estimates of rates of cancer induction per million individuals per rad, suggest that even with a postulated study of 10,000 workers followed for 25 years it is most unlikely that there would be a detectable increase in the number of cancer deaths from occupational exposure to radiation at the currently existing level. If, however, no excess mortality is demonstrated by such a study then we can conclude that the risk coefficients we assume and the levels of radiation exposure we endeavour to keep to are justified, and this information is well worth knowing.

R. H Clarke (Berkeley Nuclear Laboratories) described some work he had been carrying out in conjunction with Professor Mayneord on computer modelling of non-linear dose-response relationships. He quoted considerable published evidence for a non-linear, peaked, response between dose and risk and then discussed the implications of such a relationship in radiological protection. From his studies, Clarke was unable to show that the absolute expectations of malignant conditions were significantly greater for these non-linear dose-response functions than for the conventional linear hypothesis on which the recommendations of the International Commission on Radiological Protection are based.

The familiar film-badge which radiation workers have been used to wearing for so many years in this country will for many of them soon become a thing of the past. The National Radiological Protection Board is shortly to introduce a new themoluminescent dosemeter (TLD) for the majority of its customers and a prototype of the dosemeter was on display for the first time at the meeting. The dosemeter consists of a metal plate carrying two inserts of lithium fluoride-impregnated PTFE discs. The dosemeter is wrapped in black polythene film and worn in a black plastic holder

The advantage of a TLD is that it readily lends itself to automation in dosemeter issue and readout, and in dose record keeping. E. Greenslade (NRPB) told the meeting about the relatively simple interim dose record keeping service, based on film-badges, at present operated by the NRPB. This service covers some 3,800 workers at 330 establishments. The new TLD will form the basis of an automated dosemeter issue and record keeping service which will be capable of holding up to 200,000 records. L. Salmon (Atomic Energy Research Establishment, Harwell) described the data base system, DIRK, which had been developed at Harwell for the NRPB's automated TLD service. □

articles

Evolution of type C viral genes: evidence for an Asian origin of man

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Old World monkeys and apes, including man, possess, as a normal component of their cellular DNA, gene sequences (virogenes) related to the RNA of a virus isolated from baboons. A comparison of the viral gene sequences and the other cellular sequences distinguishes those Old World monkeys and apes that have evolved in Africa from those that have evolved in Asia. Among the apes, only gorilla and chimpanzee seem by these criteria to be African, whereas gibbon, orang-utan and man are identified as Asian, leading us to conclude that most of man's evolution has occurred outside Africa.

THE endogenous, genetically transmitted type C viruses isolated from baboons can be used to generate nucleic acid probes which provide a new approach to the study of primate evolutionary relationships[1]. With DNA transcripts of the baboon RNA virus genome, related nucleic acid sequences can be detected as normal constituents of the cellular DNA of baboons, other Old World monkeys, and apes, including man. The amount of base-pair mismatching, as determined by the thermal stability of DNA–DNA hybrids or by the final extent of hybridisation, correlates well with the classification of primates based on anatomical considerations and the fossil record[2,3]. We have explored this in more detail by comparing the virogene sequences and the overall non-repetitive cellular DNA for 11 Old World monkey and 6 ape genera, including man.

African Old World monkeys (8 genera, including several baboon species) and African apes (gorilla and chimpanzee), possess virogenes that are useful in the taxonomic classification of these animals. The Asiatic Old World monkeys (macaques, *Cynopithecus*, and langurs), and the Asiatic apes (gibbon, siamang, and orang-utan) contain virogene sequences that possess much less sequence homology (relative to the baboon) than those of the African primates. When human DNA is examined, its extent of virogene sequence divergence agrees with that obtained for the Asian apes and is substantially different from that of either African ape.

Nucleic acid homologies among primate DNAs

The extent of reassociation and the thermal stability of DNA strands derived from different species are a measure of their evolutionary relationships[4–6]. Non-repeated sequences comprise a large fraction of mammalian DNA; each sequence is believed to occur only once per haploid genome[7]. This DNA is therefore suitable for determining the extent of nucleotide diversity that has accumulated since two species diverged from their last common ancestor.

Eleven genera of Old World monkeys (Cercopithecidae) belonging to two subfamilies (Cercopithecinae and Colobinae) and six genera of apes (including man) were examined for the extent of homology of their cellular DNA with that of a baboon, *Papio cynocephalus*. The baboon is the index species for organising the Old World primates to facilitate comparison with the data obtained for the virogene sequences. Table 1 shows that the cellular DNAs of other baboon species are, as expected, most closely related to *P. cynocephalus* DNA; *P. anubis* shows the greatest sequence homology to *P. cynocephalus* while *P. papio* and *P. hamadryas* DNA possess somewhat less. The Cercopithecinae most closely related to the baboon group include the gelada, mandrill, macaques and mangabeys. Guenon, vervet and patas monkeys are more distantly related. The cellular DNAs of the more primitive subfamily Colobinae (the leaf-eating monkeys) show less nucleic acid sequence homology to baboon DNA than does the cellular DNA of any of the monkeys of the subfamily Cercopithecinae. The apes and man form a distinct group since their DNA is even more distantly related to baboon DNA than is that of any Old World monkey; the cellular DNAs of the various ape genera hybridise to approximately 50% of baboon cellular DNA with the conditions used. Three genera of New World monkeys were also examined; these are the most distantly related of the primates tested (approximately 35% homology).

The thermal stability of nucleic acid hybrids was also used as an index of the degree of base-pair mismatching between the primate DNA strands (Table 1). Mismatching results in the formation of hybrids that melt at a lower temperature; the effect of mismatched base-pairs on the thermal stability has been estimated at between 0.7 and 1.7 °C per 1% altered pairs[8]. The decrease in thermal stability between the homologous baboon cellular DNA hybrid and the hybrids formed between baboon DNA and the DNA of the other primate species is expressed as ΔT_m. It is evident that the cellular DNAs of those primates that show progressively less homology to baboon DNA also show a decrease in their thermal stability relative to the homologous baboon DNA hybrid. Therefore, the decrease in the final extent of nucleic acid homology is most likely due to an accumulation of base-pair mutations, reflecting an increasing phylogenetic distance from the baboon.

Table 2 shows the thermal stability of ape and human non-repetitive cellular DNA hybrids. Radioactively-labelled cellular DNA from diploid cultures of human, chimpanzee, and gorilla cells was hybridised to cellular DNA from cell cultures and tissues of several representatives of each species. Chimpanzee, gorilla and human cellular DNA all yield a $\Delta T_m R$ of approximately 2.3–2.6 °C when hybridised to each other. More distantly related to these three apes are the orang-utan (4.5 °C $\Delta T_m R$), the gibbon and siamang (6.3–6.5 °C $\Delta T_m R$), and the baboon (8.6–9.3 °C $\Delta T_m R$). The $\Delta T_m R$ values shown in Table 2 for ape–baboon DNA hybrids are the same as the values shown for the baboon–ape hybrids in Table 1. There is therefore a

Table 1 Nucleic acid homology and thermal stability between baboon cellular DNA and the DNA from various primate species

Species		Baboon cellular DNA	
	% hybrid	ΔT_m (°C)	$\Delta T_m R$ (°C)
Old World monkeys			
Cercopithecidae			
Cercopithecinae			
Baboon (*Papio cynocephalus*)	100	—	—
(*P. anubis*)	99	0.1	0.2
(*P. papio*)	96	0.4	0.7
(*P. hamadryas*)	93	0.7	1.1
Gelada (*Theropithecus gelada*)	90	1.0	1.8
Mandrill (*Mandrillus sphinx*)	88	1.2	2.1
Celebes ape (*Cynopithecus niger*)	84	1.2	2.3
Rhesus (*Macaca mulatta*)	86	1.4	2.7
Stump-tailed macaque (*M. arctoides*)	85	1.3	2.5
Pig-tailed macaque (*M. nemestrina*)	82	1.4	2.9
Crab-eating macaque (*M. fascicularis*)	85	1.4	2.8
Mangabey (*Cercocebus atys*)	84	1.4	2.6
(*C. torquatus*)	82	1.4	2.6
Guenon (*Cercopithecus campbelli*)	77	2.1	3.9
Vervet (*C. pygerythrus*)	75	2.2	4.4
Patas (*Erythrocebus patas*)	71	2.4	4.8
Colobinae			
Colobus (*Colobus guereza*)	64	3.2	6.5
Langur (*Presbytis obscurus*)	62	3.3	6.4
Apes			
Gibbon (*Hylobates lar*)	50	4.5	9.0
(*H. agilis*)	49	4.4	9.0
Siamang (*Symphalangus syndactyusl*)	50	4.4	8.9
Orang-utan (*Pongo pygmaeus*)	51	4.4	8.9
Chimpanzee (*Pan troglodytes*)	54	4.8	9.3
Gorilla (*Gorilla gorilla*)	50	4.9	9.5
Man (*Homo sapiens*)	56	4.7	9.2
New World monkeys			
Woolly (*Lagothrix spp.*)	31	8.2	15.2
Capuchin (*Cebus spp.*)	37	8.5	15.0
Howler (*Alouatta spp.*)	33	8.0	15.0

The DNA of a baboon lung cell strain was labelled with ^{3}H-thymidine (120 μCi per ml of cell culture media) for 72 h and extracted[20]. "Non-repetitive" cellular DNA was isolated by removing the highly reiterated DNA sequences that anneal by a C_0t of 200 (40% of the total DNA) by fractionation on hydroxyapatite[1]. Cellular DNA was extracted from tissues and cell cultures, and sonically treated to a mean size of 6–8S as determined by centrifugation on alkaline sucrose gradients[20]. DNA–DNA hybridisations were incubated at 65 °C in reaction mixtures containing 0.01 M Tris, *p*H 7.4, 0.75 M NaCl, 2×10^{-3} M EDTA, 0.05% SDS, 40,000 c.p.m. of ^{3}H-thymidine-labelled baboon cellular DNA (0.2 μg) per ml and 2–4 mg of cellular DNA per ml. Hybridisations were started by heating the mixtures to 98 °C for 10 min, cooling on ice to 4 °C, and incubating at 65 °C. At various times, 0.025-ml portions were removed and frozen at −80 °C. The percentage hybrid is the saturating normalised value obtained after digestion of the hybrids with the single-strand specific nuclease S_1 (refs 36, 37). All hybridisations were carried out to a C_0t value[7,38] of 4×10^4. The actual final extent of *P. cynocephalus* cellular DNA hybridisation to its non-repetitive cellular ^{3}H-DNA was 76%. The temperature at which 50% of the hybrids are dissociated (T_m) was 86 °C for the homologous *P. cynocephalus–P. cynocephalus* hybrid. The ΔT_m is the difference in T_m between the other DNA–DNA hybrids and the T_m of the homologous hybrid. $\Delta T_m R$ is the thermal stability difference for all the non repeated DNA[4], and is the ΔT_m value corrected for the final percentage of hybridisation of the cellular DNA to the labelled baboon DNA. This correction is based on the assumption that the difference between the various primate DNAs is due to divergence from common ancestral nucleic acid sequences, and not to the addition or deletion of large amounts of DNA unrelated to that in the species being tested.

close agreement in $\Delta T_m R$ values for the various reciprocal hybrids tested. The siamang and gibbon cellular DNAs possess as much nucleic acid sequence homology (2.5 °C $\Delta T_m R$) as chimpanzee, gorilla and human DNA share with each other.

The thermal stabilities of the various non-repetitive cellular DNA hybrids were used to construct a phylogenetic tree of primate evolution (Fig. 1). Since there is an apparent effect of generation time on the rate of accumulation of base-pair mutations in DNA[4,9,10], divergence times for certain of the apes have been adjusted for the time needed to reach a fertile state, as explained in the legend. In addition, all the divergence times shown depend on the choice of 30 Myr as the time when Old World monkeys and apes initially diverged[11]. The data show the gelada closely related to the baboon and are consistent with a model in which the baboon, mangabey and macaque lineages have been derived from a common ancestor somewhat more recently (7–8 Myr ago) than the lineage leading to the *Cercopithecus* and *Erythrocebus* group (12 Myr). The subfamily Colobinae (langur and colobus) seems to have diverged from the other Old World monkey subfamily Cercopithecinae approximately 18 Myr ago. Hybrid animals have been obtained by breeding different species of *Papio* (*P. anubis* and *P. hamadryas*, for example) and different species of *Macaca* (*M. mulatta* and *M. nemestrina*), as well as *Papio* and *Theropithecus*, and *Papio* and *Macaca*[12]. The *Papio–Theropithecus* hybrids are apparently fertile, while the *Papio–Macaca* hybrids are reported to be sterile[12]. These data support the conclusion that the *Papio–Theropithecus* divergence is more recent than that of *Papio* and *Macaca*, and suggest that a $\Delta T_m R$ difference of 2 °C for the reassociation of DNA hybrids may represent the upper limit in anthropoid primates for genetic compatibility leading to the production of fertile offspring.

As is evident from Fig. 1, man is quite closely related to the chimpanzee and gorilla. The human–chimpanzee and human–gorilla "non-repeated" cellular DNA hybrids dissociate with a $\Delta T_m R$ of approximately 2.4 °C (ΔT_m 1.3 °C) lower than the dissociation temperature obtained for the reannealing of human DNA. The divergence between chimpanzee, gorilla and man emerges therefore as a trichotomy with a common ancestor 12 Myr ago (8 Myr if the data are not corrected for the longer generation time of this group). Our data do not reveal any two of these three species as being closer to each other than the third (see Table 2). Both African apes, then, are equally close to man; our next closest relatives, but clearly more distant than the African apes, are the orang-utan (diverging 18 Myr ago) and the gibbons (diverging 26 Myr ago). The gibbon and siamang are closely related to each other, having last had a common ancestor about 6–8 Myr ago.

The data obtained correlate closely with those of other investigators who used DNA reassociation techniques[4–6,9] or amino acid and immunological data[13–17] to classify the primates, as well as with some of the phylogenetic relationships established on the basis of anatomical considerations and the fossil record[2,3,11]. The nucleic acid sequence homology of ape cellular DNA supports the classification proposed on an immunological basis by Goodman and Moore[17] that places man, chimpanzee and gorilla in one subfamily (Homininae) since they clearly had a common ancestor more recently than any of these three species had with the orang-utan or with the gibbons. The genetic distance between man, chimpanzee and gorilla as measured by the decrease in thermal stability of nucleic acid hybrids ($\Delta T_m R$) is comparable, for example, with that found between gibbon and siamang, or between baboon, rhesus and mangabey (see Table 1).

The actual evolutionary dates for the divergence of the various primate species depend on the choice of 30 Myr for the ape–Old World monkey divergence. If this date is shown to be radically wrong (most likely as an underestimate) by future palaeontological discoveries, the relative relationships among the various primate species would nevertheless remain the same. For example, the extent of nucleic acid sequence divergence among chimpanzee, gorilla and man is three to four times less than that between any of these three Homininae and the Old World monkeys.

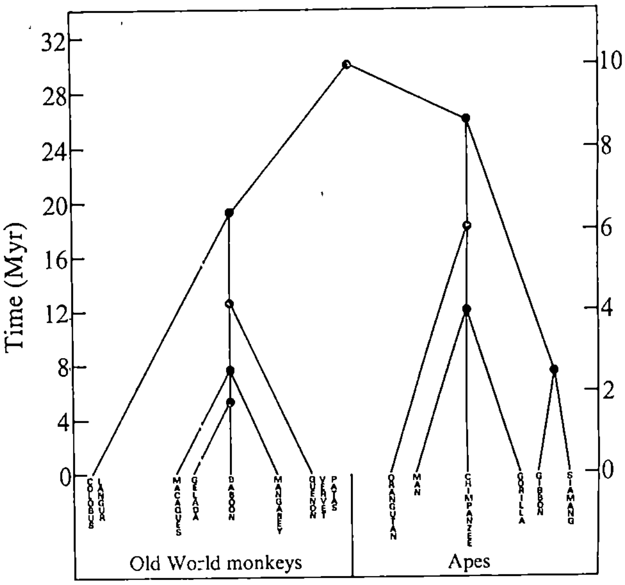

Fig. 1 Phylogenetic tree depicting relationships among the cellular DNAs of Old World monkeys and apes. The $\Delta T_m R$ (°C) of the various DNA–DNA hybrids shown in Tables 1 and 2 are plotted as a function of years of evolution. The points shown depend on the choice of 30 Myr for the time when Old World monkeys and apes diverged[11]. For the Old World monkeys (generation time 4–6 years[39]), a value of 1 °C $\Delta T_m R$ equal to 3 Myr of evolution was chosen based on the fact that the $\Delta T_m R$ between Old World monkey and ape DNA is approximately 9 °C. This relationship was also employed for the gibbon and siamang (generation time approximately 6 yr). For the longer lived higher apes, the values were adjusted as follows: orang-utan (1 °C $\Delta T_m R$ equal to 4 Myr) and chimpanzee, gorilla, and man (1 °C $\Delta T_m R$ equal to 5 Myr) (generation time of approximately 8–10 yr). The generation time is taken as the time needed to reach a fertile state; the present-day values are assumed to provide an estimate of the generation time of the ancestors of the species[40]. A more comprehensive discussion of the correction for differences in the generation times among present-day primates is in ref. 10.

Nucleic acid homologies among primate type C viral genes

All Old World monkeys and apes have previously been shown to contain endogenous primate type C virogenes as an integral component of their cellular DNA[1]. We therefore examined these primates for the extent of homology of their type C virogenes to the type C virus isolated from baboons. The baboon type C virus used in these studies was first isolated and subsequently propagated in non-primate cell lines (dog, bat, or mink)[18,19] to eliminate the possibility that the radioactively-labelled DNA probes prepared from the baboon viral RNA might contain other primate cellular sequences.

Figure 2 shows that there is a decrease in the final extent of hybridisation and in the thermal stability of hybrids formed between the baboon viral DNA probe and primate cellular DNA that is a function of phylogenetic distance from the baboon. The hybrid with the highest thermal stability is obtained with the cellular DNA of *P. cynocephalus*, the species from which the baboon type C virus was isolated. The DNA from three other species of baboons form hybrids with the baboon type C DNA probe that are slightly less stable (ΔT_m values range from 0.3 to 2.2 °C lower). The cellular DNAs from other species of Old World monkeys form hybrids that contain even more extensive base-pair mismatching. The reduced thermal stabilities obtained with the Old World monkey DNAs also indicate that the partial homologies detected between the baboon type C viral probe and the DNAs from these primates are a result of alteration by base-pair substitution or by rearrangements, of much, and perhaps all, of the viral genome, and not to the strict preservation of a large segment of the viral genome.

Table 2 Thermal stability of hybrids formed between ape non-repetitive cellular DNAs

Species	Cellular DNAs $\Delta T_m R$ (°C)			
	Chimpanzee	Gorilla	Man	Gibbon
Chimpanzee	—	2.4	2.3	6.3
Gorilla	2.6	—	2.4	6.5
Man	2.4	2.6	—	6.9
Orang-utan	NT	4.5	NT	NT
Gibbon	6.5	6.5	6.4	—
Siamang	6.3	NT	6.4	2.5
Baboon	9.3	8.6	9.0	9.1

Chimpanzee, gorilla, human and gibbon cell cultures were each labelled with ^{3}H-thymidine and the non-repetitive cellular DNA isolated and hybridised to the cellular DNA of the species listed as described in the legend to Table 1. The chimpanzee cell cultures (obtained from Dr C. J. Gibbs) were derived from brain cells. One line of chimpanzee liver cells obtained from a commercial source and used in our initial experiments characterising the baboon probe[20] has been identified as human in origin. The $\Delta T_m R$ values for the various DNA–DNA hybrids are listed. The ΔT_m values are approximately one-half the values shown; for example, the ΔT_m value for the human–chimpanzee hybrid was 1.4 °C. The temperature at which 50% of the hybrids are dissociated (T_m) ranged between 84 °C to 88 °C for the homologous DNA–DNA hybrids in various experiments. The T_m values were derived from six separate experiments in which hybrids were melted at 1 °C intervals over a range of ±7 °C from the T_m.

A striking finding, however, is that the decrease in nucleic acid homology and thermal stability for the type C virogene sequences does not always parallel the decrease in thermal stability of the non-repetitive cellular DNA. For example, even though the macaques and mangabeys are approximately equally distant phylogenetically from the baboon (2.6 °C $\Delta T_m R$; see Table 1 and Fig. 1), the virogene sequences of these animals are quite different, forming hybrids with baboon viral DNA that melt 8.2 °C and 5.0 °C lower, respectively. Even more striking are the data obtained with the Colobinae. Colobus and langur cellular DNA possess an equivalent

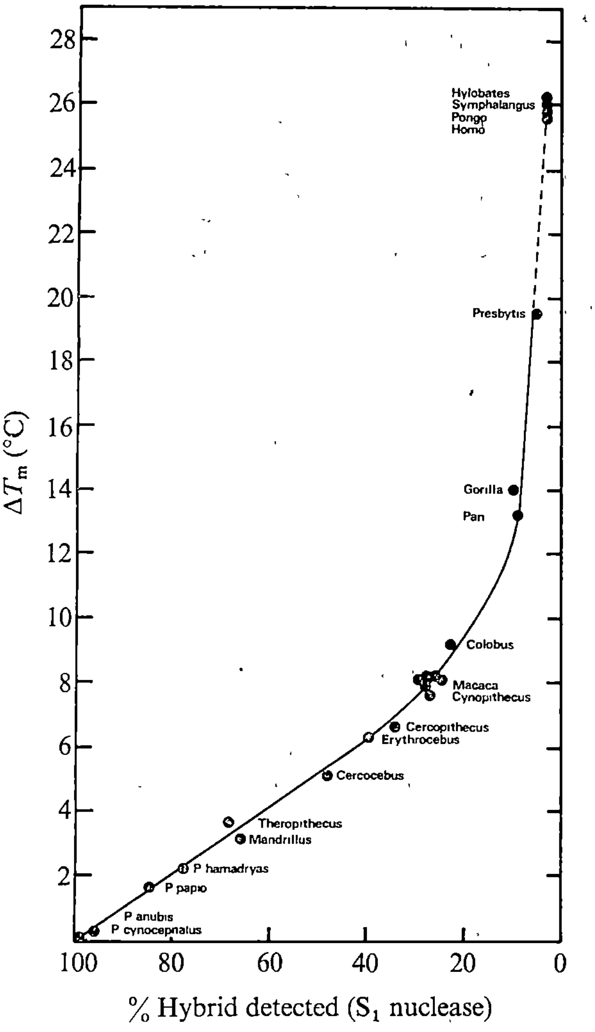

Fig. 2 Hybridisation of baboon type C viral DNA probe to primate cellular DNAs. ^{3}H-thymidine-labelled viral DNA probes were prepared as follows: the baboon viruses[18,19] were propagated in either a canine thymus (FCf2Th) or a bat lung (TblLu) cell line from which supernatant media was collected every 3 h for 1 d and stored at 2 °C. The supernatant was clarified of cellular debris and the virus banded on sucrose gradients, pelleted, and used immediately in an endogenous reverse transcriptase reaction. Reaction mixtures were incubated at 37 °C for 2 h and contained 0.04 M Tris, pH 7.8, 0.06 M KCl, 6×10^{-3} M magnesium acetate; 2×10^{-3} M dithiothreitol; 0.02% (v/v) Triton X-100; 30 μg ml^{-1} actinomycin D; 5×10^{-5} M ^{3}H-TTP (50 Ci mmol^{-1}), and 2×10^{-3} M each of dATP, dCTP, and dGTP. These conditions have been shown to result in the synthesis of long, representative DNA copies of the viral genome[41–43]. The ^{3}H-DNA baboon probe was deproteinised and purified as described previously[20]. The specific activity of the ^{3}H-DNA was 1.8×10^7 c.p.m. μg^{-1}. The ^{3}H-DNA probes contained 65–86% of their respective 70S viral RNA sequences at a ^{3}H-DNA: ^{32}P-viral RNA molar ratio of 1.0 (ref. 20), indicating that they contain most of the sequences present in viral RNA and that these sequences are in proportions similar to their content in 70S RNA. Several of these baboon probes were subsequently further purified by hybridising them to 70S or 35S viral RNA extracted from baboon virus grown in the cells of a non-primate species different from the one in which the virus had been grown. Probes prepared from the baboon virus grown in the bat lung cell line were cycled against RNA extracted from the virus grown in the canine thymus cell line. In some experiments the reciprocal cycling was performed: virus grown in the canine thymus cell line was cycled against RNA extracted from the virus grown in the bat cell line. The non-hybridised portion of the baboon ^{3}H-DNA probe was removed by digesting with S_1 nuclease. These probes were then treated with alkali to hydrolyse the RNA, and deproteinised, and purified as described[20]. Viral probes treated in this manner hybridised to 90–100% of baboon viral RNA. DNA–DNA hybridisations were incubated as described in the legend to Table 1, and the extent of hybridisation determined with the single-strand specific nuclease S_1. The normalised final percentage values obtained after hybridising the various primate cellular DNAs to the baboon viral probe are shown. The ΔT_m values obtained for the DNA–DNA hybrids relative to the homologous baboon viral ^{3}H-DNA–*Papio cynocephalus* cellular DNA hybrid are listed on the ordinate. The final percentage hybridisation values of the baboon viral probe to gibbon, siamang, orang-utan and human DNA are too low (3–4%) (see Table 3) for the ΔT_m to be determined experimentally; these values have been extrapolated from the curve as shown.

amount of nucleic acid sequence dissimilarity relative to baboon cellular DNA (6.4–6.5 °C ΔT_mR, Table 1), but the virogenes of these two species hybridise to 23% and 6% of the baboon viral probe, respectively (Fig. 1, Table 4). Finally, although the DNA of all six genera of apes are indistinguishable relative to baboon cellular DNA (8.9–9.5 °C ΔT_mR), two of the apes, chimpanzee and gorilla, contain virogene sequences that are much more closely related to baboon virogene DNA than are those of the other apes (gibbon, siamang, orang-utan) or man. In fact, chimpanzee and gorilla cellular DNA consistently hybridise more to the baboon virogene probe than does the DNA of the langur, an Old World monkey. The hybridisation values of gibbon, siamang, orang-utan and human DNA to baboon viral DNA as detected by S_1 nuclease are too low (3–5%) for the ΔT_m values to be determined experimentally; they are extrapolated from the curve as shown in Fig. 2.

Earlier studies on primate evolution using the baboon viral probe[1,20,21] focused on the observation that the baboon virus was, in fact, an endogenous primate virus and that related nucleic acid sequences could be detected in various other Old World monkey tissues. The final extents of hybridisation to ape DNAs were much lower than those obtained to Old World monkey DNAs but significantly higher than those obtained to New World monkey, prosimian, or other mammalian DNA. The differences in final extent between the various ape DNAs were difficult to determine based on only a few S_1 nuclease determinations[1]. In one previous study, the source of chimpanzee DNA was a tissue culture line (chimpanzee liver cells, obtained from Flow Laboratories, Rockville, Maryland) which provided an apparent discrepancy in the data[20]. Further analysis revealed the cell line DNA to be human, most probably HeLa, and not chimpanzee.

Since only a low degree of homology is obtained with the baboon viral probe to ape DNA using stringent hybridisation assay conditions (such as S_1 nuclease), a large number of experiments is needed to obtain statistically significant differences. The final extent of hybridisation (from several separate experiments) of the baboon viral probe to colobus and langur DNA and to chimpanzee, gorilla, gibbon, orang-utan and human DNA is shown in Table 3. Although the final hybridisation levels obtained with langur, gibbon, and human DNA are low, they are significant when compared with the hybridisation values obtained with four non-primate mammals (cow, hippopotamus, pig and rabbit) as well as with the non-mammalian salmon sperm DNA. The DNAs from a total of 36 human tissues have been separately examined. Most show a small increase in the final extent of hybridisation when compared with non-primate DNAs, some do not hybridise significantly above the levels found with the non-primate controls, while still others yield what seem to be unexpectedly high final hybridisation values to the baboon viral transcripts. The latter DNAs (3 of 36) have been obtained from tissues of individuals with tumours. The basis for the apparent occasional "higher reactors" among human DNA samples requires further study and will be the subject of a separate report. Nevertheless, the large differences in the final extent of hybridisation of colobus and langur DNA and of chimpanzee and human DNA to the baboon type C viral probe are clearly evident. With a DNA transcript prepared from an endogenous domestic cat virus (RD-114) that is partially related to the baboon virus, a higher final extent of homology has also been obtained to gorilla and chimpanzee DNA than to human or gibbon cellular DNA[22].

Hybrids with more extensive mismatching can be detected by lowering the stringency of the annealing conditions[23]; this permits the detection of partially homologous sequences in more distantly related species[4]. Table 3 shows data obtained with the baboon viral probe when the hybridisation conditions are modified by decreasing the temperature to 56 °C, the salt concentration to 0.50 M Na^+, and by using hydroxyapatite rather than S_1 nuclease, thus allowing partial hybrids to be detected as double-stranded molecules. Although the background values obtained with non-primate DNA increase, the higher final extent of hybridisation to chimpanzee and gorilla DNA compared with gibbon, orang-utan and human DNA are still evident.

A higher final extent of hybridisation to the various Old World monkey DNAs can also be obtained by changing the S_1 nuclease incubation conditions. For example, by decreasing the temperature of the S_1 nuclease incubation from 45 °C to 37 °C and by increasing the monovalent cation concentration from 0.15 M to 0.30 M, the final extent of hybridisation of the baboon viral probe to mangabey DNA increases from 50% to 85%, and the value to rhesus and other macaque DNAs increases from 25% to 60%. The relative relationships among the various primate virogene sequences remain the same.

Differences in African and Asian primate type C viral genes

The Old World monkeys and apes inhabit both Asia and Africa. All the genera of the Old World monkey subfamily Cercopithecinae inhabit Africa with the exception of various macaque species and the closely related celebes ape (*Cynopithecus*), which inhabit Asia. Table 4 lists representative species from the subfamily Cercopithecinae that have evolved in both areas and whose DNA has diverged to a comparable extent from baboon cellular DNA (as shown by their $\Delta T_m R$ values). The cellular DNA of those animals whose habitat is Africa hybridises to a much greater extent to the baboon viral DNA probe. The DNA extracted from two species of mangabeys, for example, hybridises 48% and 51% to the baboon type C viral DNA probe, whereas the DNA extracted from four different species of macaques hybridises only 25–28%. The use of hydroxyapatite to detect these hybrids raises the final extent of hybridisation to 65–70% for mangabey DNA and to 45–50% for rhesus DNA, but the clear differences between these primates remain. Two genera from the other Old World monkey subfamily, the Colobinae, were also examined. The cellular DNA from the African species, the colobus, hybridises to 23% of the baboon virogene probe, whereas the Asian langur cellular DNA hybridises to only 5% of the probe. Finally, among the apes, the cellular DNA of chimpanzee and gorilla (which inhabit Africa) hybridise 9% and 10%, respectively, to the baboon virogene probe. The gibbon, siamang and orang-utan, which inhabit Southeast Asia, hybridise to a much lower extent to the baboon viral probe, as does the human DNA.

Table 3 Detection of endogenous type C virogene sequences in various primate and human tissues

Species	No. of individuals tested	No. of tissues tested	No. of Expts	% Hybrid S_1 nuclease* Avg.†	s.d.	% Hybrid minus background‡
Colobus	2	3	18	27.8	±2.6	24.1
Langur	1	2	12	9.1	±1.5	5.4
Gorilla	2	5	15	14.0	±1.4	10.3
Chimpanzee	5	11	44	13.5	±3.1	9.8
Gibbon	8	14	28	6.8	±1.3	3.1
Man	36	36	68	7.0	±2.6	3.3
Cow	3	3	14	3.5	±1.0	0
Hippopotamus	1	2	6	3.8	±0.8	0
Pig	5	10	18	4.3	±0.9	0
Rabbit	2	6	12	3.3	±0.9	0
Salmon	1	1	10	1.5	±0.7	0
				% Hybrid hydroxyapatite ‖		
Chimpanzee	2	3	6	26.7	±4.7	18.7
Gorilla	1	2	4	27.8	±2.5	19.8
Gibbon	2	2	6	15.2	±2.0	7.2
Orang-utan	1	1	4	14.7	±4.8	6.7
Man	5	5	12	15.8	±3.1	7.8
Cow	1	1	12	8.0	±2.1	0
Pig	2	2	5	8.5	±2.2	0
Rabbit	1	2	12	7.5	±2.0	0
Salmon	—	—	5	2.7	±0.7	0

*Cellular DNA was extracted from various tissues of the species listed[20]; salmon sperm DNA was obtained commercially. ^{3}H-DNA probes prepared from the baboon type C virus grown in a bat lung cell line (TblLu) or in a canine cell line (FCf2Th) were hybridised at 65 °C in 0.75 M NaCl to the various cellular DNAs as described in the legend to Table 1. The extent of hybridisation was determined by digestion of the hybrids with S_1 nuclease[36,37].

†The average hybridisation values from the number of determinations listed are normalised to the actual final extent of hybridisation obtained to baboon (*P. cynocephalus*) cellular DNA (86–100%). Cycled viral probes (see Fig. 2) and uncycled probes yielded comparable hybridisation values to the various species when normalised to the homologous system. Some baboon viral probes prepared using virus produced by human cells, however, gave artefactually high values with human cellular DNA. The standard deviation of all the data is shown in the next column.

‡The values obtained after hybridising the baboon viral probe to the primate cellular DNAs are shown; the average hybridisation value obtained with non-primate mammalian DNA has been subtracted. Non-mammalian DNA (salmon sperm) yields an even lower hybridisation value than that obtained for the non-primate mammals; this value has not been included in the average.

‖ The extent of hybridisation between the ^{3}H-DNA probe prepared from the baboon type C virus and the cellular DNAs was detected on hydroxyapatite[1]. The percentage hybridisation values shown are normalised values from four to seven experiments; the final extent of hybridisation to baboon cellular DNA varied between 74–88%. DNA–DNA hybridisations were performed at 56 °C in 0.025 ml reaction mixtures containing 0.01 M Tris, pH 7.4, 0.50 M NaCl, 2×10^{-3} M EDTA, 0.05% SDS, 2,000 c.p.m. of ^{3}H-baboon type C DNA probe, and 0.1 mg of cellular DNA.

Table 4 Differences in extent of genomic alteration between African and Asian Old World monkey and ape virogenes

	African origin				Asian origin		
Primate	$\Delta T_{\rm m}R$ (°C)* cellular DNA	No. of species	% Hybrid† virogene DNA	Primate	$\Delta T_{\rm m}R$ (°C)* cellular DNA	No. of species	% Hybrid† virogene DNA
				Old World monkeys			
				Cercopithecinae			
Mandrill	2.1	1	66	Celebes Ape	2.3	1	27
Mangabey	2.6	2	48, 51	Macaques	2.5–2.9	4	25–28
				Colobinae			
Colobus	6.5	1	24	Langur	6.4	1	5
				Apes			
				Gibbon	9.0	1	3
Gorilla	9.5	1	10	Siamang	8.9	1	3
Chimpanzee	9.3	1	9	Orang-utan	8.9	1	3
Man	9.2	1	3	Man	9.2	1	3

*The $\Delta T_{\rm m}R$ (°C) of hybrids formed between the cellular DNA of the primate species and ^{3}H-non-repetitive baboon cellular DNA is shown. These values are from the data listed in Table 1.

†The normalised final percentage of hybridisation of primate cellular DNA to the baboon viral DNA probe is listed These values are from the data shown in Fig. 2 and Table 3. By varying the S_1 nuclease incubation conditions a higher final extent of hybridisation to the various primate DNAs can be obtained. For example, by increasing the monovalent cation concentration from 0.15 M to 0.30 M and by decreasing the temperature of the S_1 nuclease incubation from 45 °C to 37 °C, the final extent of hybridisation of the baboon viral probe to mangabey DNA increases from 50% to 85%, and the value to rhesus and other macaque cellular DNAs increases from 25% to approximately 60%. The substantial difference between the various African and Asian primate DNAs remains, however.

The hybridisation data obtained with non-repetitive cellular DNA group the primates in terms of their phylogenetic proximity to the baboon (Fig. 3). The data obtained with the endogenous type C virogene sequences show that the type C virogenes of the Asian primates hybridise less to the baboon probe than do the virogenes of their African counterparts. Thus, with the endogenous primate type C viral gene sequences (although not with the overall cellular DNA), we are able to discriminate quite clearly between Asian and African primates. Among the apes, the gibbon and orang-utan clearly stand out as Asian. Within the ape subfamily Homininae (man, chimpanzee and gorilla[17]), *Homo sapiens* stands out as the only non-African representative. We therefore propose an Asian origin for man, with a long history in that region before our species migrated all over the world.

An alternative interpretation of the data would be that man is unusual among primates in having a deletion (or series of deletions) in his virogene sequences that makes its properties coincide with those of the orang-utan, gibbon and siamang. A partial deletion hypothesis in man, however, does not account for the marked differences in the extent of genomic alteration between the Asian and the African Cercopithecinae or Colobinae virogenes when tested with baboon viral DNA transcripts.

The results presented here show that the extent of nucleic acid dissimilarity between the primate virogenes depends on two factors: (1) the taxonomic distance of the species from *Papio*, and (2) the geographical habitat of the species. A comparison of baboon and mangabey type C virogenes, which have evolved together in Africa, and of baboon and macaque virogenes, where the species have been physically separated from each other for several million years, reveals that the differences in their virogene sequences result from the effect of one environment as opposed to another. Thus the differences in thermal stability and final extent of hybridisation in this particular set of mammalian genes is influenced by environmental factors. The ubiquitous presence of endogenous type C virogenes among anthropoid primates and their evolutionary persistence for at least 30–40 Myr suggest that such genes provide functions with a selective advantage to the species possessing them. Thus, some functions of the type C virogenes are acted on by the environment in such a way as to select for certain genetic sequences and against others.

Type C viruses can be isolated readily from four different species of baboons (*P. cynocephalus*, *P. anubis*, *P. papio*, and *P. hamadryas*) and from the closely related genus, *Theropithecus*[18,19,24,25], all of which inhabit Africa. Extensive attempts to isolate infectious type C viruses from other anthropoid primates using the same techniques that have led to the isolation of the baboon and gelada viruses, have so far been unsuccessful[25]. The action of selective environmental pressure presupposes that the virogenes are expressed during the lifetime of the animal and, further, that they affect the survival or the reproductive capacity of individuals of the species. The primate species from which infectious viruses have not yet been isolated must therefore express at least some virogene products on which the environment may act. Resistance to infection by exogenous type C viruses might be a critical selective factor; baboons range throughout Africa and release a virus that might be potentially damaging to recipient species. The endogenous domestic cat virus RD-114 has been postulated to have originated from an endogenous Old World monkey type C virus horizontally transmitted to an ancestor of the domestic cat[22]. This suggests the possibility that ancestors of the baboon may have been releasing infectious virus particles for several million years. The selective pressure on the other primates to maintain their related virogenes and so protect themselves against viral infection would be continuous and strong. Endogenous type C virogenes have previously been shown to restrict the replication of highly related viruses in the same cells[26]. The African primates may well have had to conserve a greater degree of nucleic acid sequence homology relative to the baboon type C virus in order to restrict the replication of this virus in their cells. Those portions of the virogene most directly involved in controlling virus spread from animal to animal would be expected to show the most extreme environmental effect.

Asian primates, which would not be exposed to the baboon endogenous virus, would be under less selective pressure to maintain cellular nucleic acid sequences similar to those present in the baboon type C viruses; their virogenes, then, would be free to accumulate genomic alterations at an apparently faster rate relative to baboon virogene sequences. Rhesus monkey cells, for example, are extremely permissive for the replication of baboon type C viruses, whereas baboon, gelada and mangabey cells restrict baboon virus replication. Man, postulated here to be an Asian primate that has only recently migrated into Africa, would, therefore, be expected to be susceptible to infection by baboon type C virus. Human cell

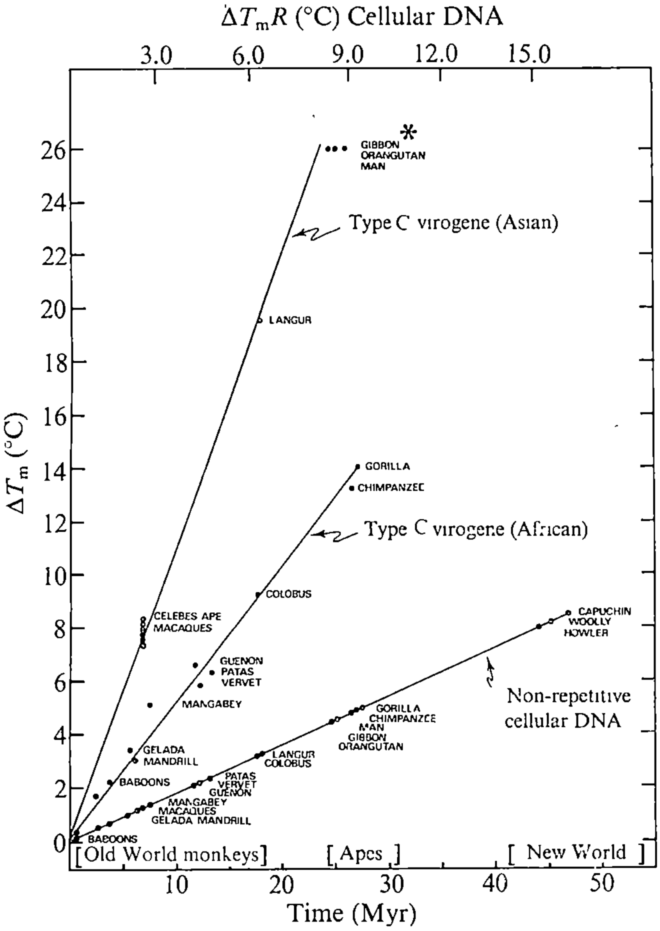

Fig. 3 Relationship among primate cellular DNAs as determined by the extent of their homology to baboon non-repetitive cellular DNA and to the baboon type C viral DNA probe. The genetic distance from the baboon (*P. cynocephalus*) of various primate species as determined by the thermal stability (ΔT_mR) of hybrids formed between their cellular DNA and non-repetitive baboon DNA is shown in the lower curve. The thermal stabilities of the virogene sequences of all the African primates tested fall on the middle curve and those of all the Asian primates and also of man fall on the upper curve. No direct comparison can be made between the rate of base-pair changes in the cellular and viral nucleic acid sequences since the latter are present in 10–20 copies per haploid genome[44] whereas the cellular gene sequences are predominantly present in only one copy[7].

*Accurate T_m values for the hybridisation of gibbon, orangutan and human cellular DNA to the baboon viral probe have not been determined experimentally; they are extrapolated from the data shown in Fig. 2. Nevertheless, these T_m values are lower than those obtained to gorilla and chimpanzee DNA.

cultures, in fact, have been shown to be highly susceptible to infection by various isolates of the baboon type C virus[18,19,25].

The rhesus monkey produces endogenous type C viruses that can be seen by electron microscopy[27], and expresses type C viral p30 antigen and reverse transcriptase[28,29], but so far, no infectious virus has been isolated. Such a virus would allow us to study Asian primate virogenes and perhaps determine whether they too are more closely related to one another than they are to the virogenes of African primates.

Possible implications for the origin of man

Because of man's close morphological and anatomical resemblance to the African apes, and because most of the recent palaeontological discoveries have been made in Africa, many investigators have proposed an African origin for our species. The results presented here lead to a conclusion that is not consistent with the palaeontological studies which place the major developmental changes that have led to modern man as occurring in Africa; rather, they suggest to us that man has originated outside Africa.

The time of divergence of the hominid stock from the apes has variously been estimated at between 4 and 20 Myr; our data indicate a divergence time of approximately 12 Myr. *Ramapithecus* and *Kenyapithecus*, which have been dated by potassium–argon at 12–14 Myr were found in India and in East Africa[30–32]. They seem, according to our data, to precede or perhaps to be contemporary with the man–chimpanzee–gorilla ancestor. There then follows a period of about 10 Myr, virtually all of the Pliocene, where fossil finds have been few and have not been extensively characterised. Between 1 and 3 Myr, fossils that appear to be hominids have again been found both in Africa and Asia. Our data suggest that man's direct ancestors probably spent most if not all of the Pliocene in Asia. The older Australopithecines found in Africa, though clearly hominids, were probably, therefore, not in the main lineage to man, but rather, unsuccessful offshoots whose progeny have not endured to the present[33]. The data do not preclude a more recent migration, perhaps in the last 2–3 Myr, of hominids into Africa. The nucleic acid sequence changes we are measuring are the result of millions of years of evolution. The relatively recent migration, or return, of man's ancestors to Africa does not seem to have reversed the long evolutionary history of man's type C virogenes while in Asia. The much more recent migrations by modern man all over the world and his divergence in the last hundred thousand years into the various races are also events which are too recent to be detected with these techniques[34,35].

Of all the primates, the hominids have been the most successful at adapting to a terrestrial life and have migrated all over the world; their geographical origin, therefore, is more obscure than that of the other primates. The major selective pressures that influence the evolutionary history of a species are recorded and retained in its genetic material. The baboon viral gene sequences have allowed us to distinguish clearly between Asian and African primates and to study the imprint of this environmental selective pressure on primate cellular DNA. The data obtained with the primate virogenes suggest that, during the Pliocene, man's ancestors were developing in Asia.

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Rare earth, Fe and Ti variations along the Galapagos spreading centre, and their relationship to the Galapagos mantle plume

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Rare earths, Fe and Ti concentrations in basalts dredged along the Galapagos spreading centre reveal that a juvenile mantle plume is welling up beneath the Galapagos Islands and mixing with the depleted asthenosphere to the north-east and north-west beneath the ridge. Unusually extensive shallow-depth, crystal fractionation is also apparent on the ridge between 85 and 87° W. The 'magnetic telechemistry' hypothesis is further clarified.

THE plate tectonic theory provides a simple and unified explanation for surface geological features such as mountain ranges, trenches, island arcs and mid-ocean ridges, and describes their kinematic relationships. The theory does not, however, adequately explain shallow oceanic platforms such as those found around the Azores, Hawaii, Iceland, and the Galapagos Islands.

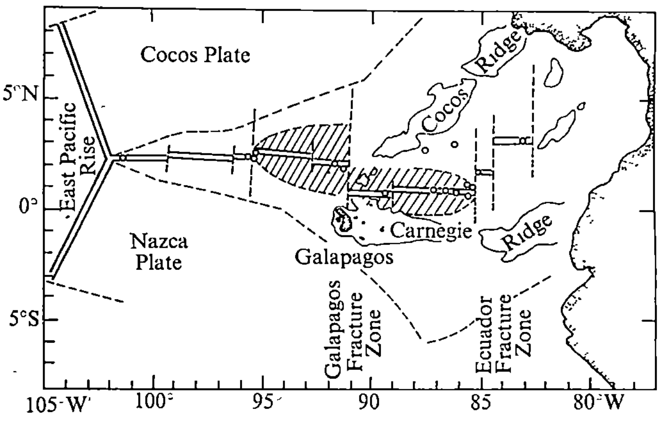
These unusually extensive outpourings of lava have been attributed to fixed hot spots[1] and mantle plumes[2]. Vogt has suggested[3] that flows of asthenospheric partial melts associated with mantle plumes could be monitored for hot spots located near the mid-ocean ridge, and that the flow is probably concentrated in a pipe-like region below the spreading axis; transform faults would tend to dam such sub-axial flow[4,5].

Schilling[4,6] has mapped large-scale chemical gradients from basalts dredged from the axis of the Mid-Atlantic Ridge away from Iceland or the Azores, and attributed the gradients to binary mixing, at shallow depth beneath the ridge, of two geochemically distinct mantle slush components: namely a mantle plume enriched in light rare earths (RE) and radiogenic Sr and Pb isotopes, and a widespread asthenosphere depleted in light RE and low Sr and Pb isotopic ratios[4,6–10] (subsequently referred to as depleted low velocity layer—DLVL).

We report here new geochemical data for the Galapagos spreading centre and its associated platform (Fig. 1). The area contains most of the typical elements of the Iceland type of 'hot spot'[11]. The Galapagos Archipelago is mainly built of tholeiitic lava of 'plume'-derived or 'blob'-derived composition, judging from their RE patterns[12–14]. The Cocos and Carnegie aseismic ridges branch from the platform, and the Galapagos spreading centre shoals where it passes north of the platform (Figs 1 and 2). No regional free-air gravity high, relative to the figure of hydrostatic equilibrium, has been observed over the region, however. Unusually high magnetic anomaly amplitudes have also been noted over the crest of the Galapagos spreading centre within 5° of the platform (Fig. 1).

Vogt and Johnson[16] have attributed the large magnetic anomaly to an oceanic crust rich in Fe and Ti derived from a mantle plume welling up beneath the Galapagos Islands and flowing outwards at shallow depth; Anderson, *et al.*[17] also suggested Fe and Ti enrichment, but suggested

Fig. 1 Location of the dredge hauls studied (circles) along the Galapagos spreading centre (double lines), and their relationships to the high magnetic amplitude region (hatched area) and major tectonic elements, namely: the Cocos and Carnegie aseismic ridges; the Galapagos Islands (blackened); fracture zones (heavier vertical dashed lines); the Galapagos Gore (light dashed line)[11,32].



that it is caused by a large 'melting anomaly' centred about the Galapagos Archipelago, with exceptionally intense, shallow-depth, fractional crystallisation of plagioclase feldspar and olivine from a normal, light RE-depleted mid-ocean ridge basaltic melt. Relative RE patterns of the Fe-rich residual liquids produced by the latter mechanism, although enriched as a whole, would remain depleted in light RE elements, and should develop an anomalous Eu depletion (proportional to the extent of plagioclase removal and anorthite content)[13,18], whereas mantle plume basalts are commonly enriched in light RE elements, and generally show no Eu anomaly. The RE distinction can thus serve as a simple test of the two mechanisms and origins proposed for the region of high magnetic amplitude over the Galapagos spreading centre.

Results from this pilot study show the effect of local Fe enrichment caused by the shallow plagioclase–clinopyroxene and olivine crystal fractionation of DLVL-derived basalts. They also indicate mantle-plume mixing along the ridge segment nearest to the Galapagos Archipelago. Extensive shallow crystal fractionation seems to be limited to 85–87°W along a segment of the ridge apparently not affected by the Galapagos mantle plume.

Results

Locations of the dredged rocks are shown in Fig. 1. The RE, FeO* and TiO_2 concentrations, and FeO*/FeO*+MgO ratios of these rocks are given in Table 1. Major element analyses and magnetic properties of rocks from stations T6, HFD-1, D-7 and D-17 have already been reported by Anderson, *et al.*[17]. The complete major element analyses of rocks from USNS De Steiguer dredge hauls are presented in Table 2. These samples were obtained on either side of the boundary delineating the high magnetic anomaly province, to define the sharpness of the transition. Cocotow dredged rocks were obtained with R. V. Melville, and their major element chemistry will be reported elsewhere (J. W. Hawkins, unpublished). The chemistry of basalt RC-10-56 has already been reported[19]. Highly differentiated and atypical oceanic volcanic rocks were also dredged north of

Fig. 2 *a*, La/Sm concentration ratio (normalised to chondrites); *b*, TiO_2 weight percentages; *c*, FeO*; in basalt erupted along the Galapagos spreading centre (data from Table 1); *d*, variations along the ridge axis of the central magnetic anomaly; *e*, water depth. Later data from Anderson, *et al.*[17]. For comparison, dotted-dashed lines are averages of 13 dredged basalts from the East Pacific Rise between 0 and 19°S (ref. 22), (FeO* = 9.2±0.5 weight %; TiO_2 = 1.41±0.22 weight %; and [La/Sm] = 0.60±0.12). The averages exclude the three ferrobasalts also reported for this normal EPR segment. The error bars attached to the averages are for 1 s d. Open symbols, dredge stations off the ridge axis; solid symbols, stations on the axis.

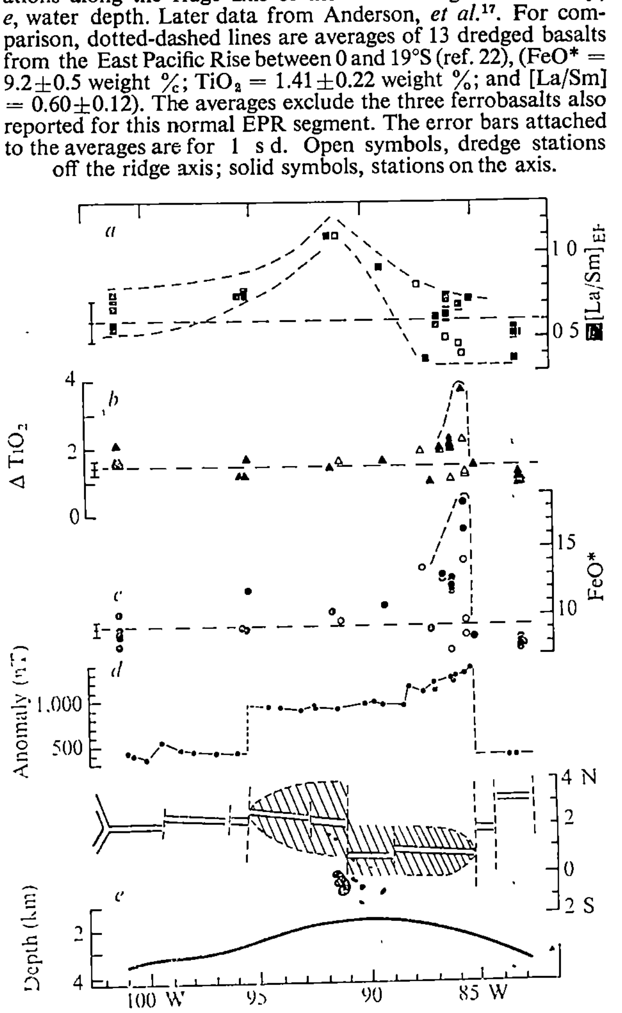


Table 1 Abundances of rare earths (p.p m), FeO* and TiO_2 (weight %) of dredged basalts from the Galapagos spreading centre†

Cruise	Station	Latitude	Longitude	Depth (m)	La	Ce	Nd	Sm	Eu	Tb	Tm	Yb	Lu	[La/Sm]$_{EF}$	FeO*	TiO_2	FeO*/FeO*+MgO
Southtow 7	D-7-1	3°12.0′N	83″02.4′W	2,502	1 3			1.8	0.64			2.0	0.32	0.52	8.30	0.87	0.525
	D-7-2	3°12.0′N	83″02.4′W	2,502	1.8			2.3	0.88			1.8	0.35	0 55	7.88	1.03	0.476
	D-7-3	3°12.0′N	83°02.4′W	2.502	1.1			2.1	0 78	0.51		2.3	0.34	0.37	7.59	1.02	0.478
	D-7-4	3°12.0′N	83°02 4′W	2.502	1.9			2.6	0.96	0.69		2.5	0.32	0.52	7.60	1 10	0.481
	D-7-5	3°12.0′N	83°02.4′W	2,502	2.1			2.9	1.1	0.77		3 1	0.37	0.51	7.71	1.23	0 557
	D-17-1	0°49.2′N	86°07.8′W	2,600	5 3			5.8	1.8	1.4		4.3	0.81	0.65	12 22	2.05	0.671
	D-17-2	0°42.9′N	86°07.8′W	2,600	7 0			6.5	2.0	1 4		6.0	0.92	0.75	12.39	2.14	0 683
	D-17-3	0°49.2′N	86°07 8′W	2,600	4.9			5.3	1.7			5.6	0.79	0.65	11.82	2.04	0.653
	D-17-4	0°49.2′N	86°07 8′W	2,600	4.8			5.2	1.7	1.3		6.3	0.80	0 63	11.90	2.01	0.653
	D-17-5	0°49.2′N	86°07 8′W	2,600	6 8			6.6	2.0	1.6		5.5	0.88	0.72	12.38	2.15	0 706
	HFD-1	3°00.0′N	86°00.10′W		1.6			2.2	0.87	0.44		2.3	0.35	0 49	7 46	1 00	0.470
Bartlett	T6-378	2°12 0′N	101°25′W	3,300–4,790	4.3			5.4	1.8			4.3	0.85	0 56	10.32	2.07	0 618
	T6-269	2°12.0′N	101°25′W	3,300–4,790	3 4			4.1	1.5			3.6	0.50	0.58	7.83	1.54	0.556
	T6-384	2°12.0′N	101°25′W	3,300–4,790	3.3			3.3	1.3			3.2	0.43	0.68	8.91	1 55	0.560
	T6-285	2°12.0′N	101°25′W	3,300–4,790	3.5			3.5	1.3			3.2	0.46	0.71	8.76	1.54	0.557
	T6-372	2°12 0′N	101°25′W	3,300–4,790	4.5			4.2	1.3			3.3	0.50	0.76	8.81	1 56	0 560
De Steiguer 41	D1A	0°42.6′N	85°30 00′W	2,377	9.8	31.9	29.0	10.2	2.9	2.6	1.8	10.7	1.5	0.68	18.36	3.62	0 809
	D1B	0°42.6′N	85°30 00′W	2,377	7.2	22.2	20.9	7.4	2.5	2.0		8.1	1.2	0.68	16.34		
	D2A	1°44.4′N	85°07.2′W	2,798	3 8	13.4	10.3	3.6	1.3	0.86	0.51	3.2	0.44	0.73	8.47	1.41	0.495
	D3A	1°02 3′N	85°25.4′W	1,554	1.5	7 1	6.1	2 6	1.0	0.79	0.56	3.1	0.45	0 39	9.59	1 23	0.608
	D3B	1°02.3′N	85°25.4′W	1,554	1.6	5.1	6.6	2 8	1.0	0 79	0.50	3.0	0.46	0 40	8.53	1 21	0.565
	D4	1°08.6′N	85°30 00′W	2,926	3 5	13.6	13.7	5.4	1.6	1.4	0.96	5.4	0.79	0.46	13 98	2.16	0.710
	D5	2°26 4′N	95°37.2′W	2,743	2.8	8.6		2.6	0 93	0.66	0.40	2.6	0.38	0 75	9.10	1.10	0.503
	D7A	2°25.2′N	95°24.6′W	2,103	2 8	9.1	6.7	2.7	0 96	0.72	0.48	2.7	0 38	0.75	9.04	1.13	0.527
	D8A	2°36 0′N	95°19.8′W	3,109	4.5	13.5	10.7	4.0	1.4	1.1	0.81	4.4	0.61	0.78	11.96	1.62	0.614
Cocotow 4	6D-1	0°51.1′N	86°32 8′W	2,330–2,432	4.6	16.4	13.5	5.2	1.7	1.3	0.94	5.4	0.80	0.62	12 77	1 89	0.654
	6D-2	0°51.1′N	86°32.8′W	2,330–2,432	3.8	12.9	11.8	4.7	1 7	1.3		5.1	0.75	0 57	12.95	1.87	0 667
	7D-1	0°51 8′N	87°05.1′W	2,385–3,230	1.1	5 8	4.8	2.1	0.90	0.71	0.50	2 7	0 32	0.37	8.98	0.94	0.504
	8D-1	2°38.4′N	87°29.1′W	2,347–3,170	5 5	17.5	12.0	4.8	1.7	1.2	0 84	4 7	0 71	0.81	13.53	1.86	0 686
	9D-1	0°46.7′N	89°13 7′W	1,777–1,845	4.9	13.3		3 7	1.4	0.96	0.63	3.0	0 45	0 91	10 72	1.54	0.592
	10D-1	1°59 9′N	91°36 6′W	1,981–2,050	5 1	13.8	10.9	3.4	1.3	0.84	0.66	3.2	0.52	1.1	10.29	1.34	0 575
RC-10-56		1°48.6′N	91°15.3′W	2,082	5.8	19.1	13.9	3.6	1.4	0.88	0.51	3.1	0.45	1.1	9 62	1.60	0 568
JB-1 Split No. 8‡					39.4 ±6.8%	75.6 ±8.1%	29.5 ±9.1%	5.4 ±9.2%	1.6 ±6.2%	1.2 ±8.3%		1.9 ±21.0%	0.30 ±3.3%				

†RE patterns were obtained using instrumental neutron activation analysis (R. Kingsley); FeO* (total iron as FeO), TiO_2, and MgO by wet chemical analyses (R. Evans)
‡Basalt standard JB-1, average of 5 replicate analyses and 1 standard deviation (%).

Table 2 Major element analyses of dredged basalts from De Steiguer Cruise 41 (Galapagos spreading centre)*

Sample No.	D1A	D2A	D3A	D3B	D4	D5	D7A	D8A
SiO_2	48.43	49.55	50.95	51.39	50.49	49.76	50.15	50.23
Al_2O_3	11.51	16.58	15.84	16.11	13.14	15.71	15 87	14.22
Fe_2O_3	3.61	1.15	2.70	1.92	4.03	1.17	1.44	1.74
FeO	15.11	7.44	7.16	6.80	10.35	8.05	7.74	10.39
MgO	4.33	8.65	6.18	6.57	5.72	9.00	8.12	7.52
CaO	8.95	11.57	12.46	12.61	10.38	12.09	12.27	11.06
Na_2O	2.66	2.77	2.33	2.33	2.55	2 26	2.22	2.34
K_2O	0.24	0.08	0.15	0.12	0.06	0.07	0.11	0 14
H_2O^+	0.91	0.21	0.49	0.45	0.33	0.21	0.38	0.36
H_2O^-	0.17	0.04	0.29	0.21	0.38	0.09	0.14	0.09
TiO_2	3.62	1.41	1.23	1.21	2.16	1.10	1.13	1.62
P_2O_5	0.32	0.21	0.11	0.11	0.22	0.11	0.11	0 19
MnO	0.26	0.15	0.17	0.16	0.24	0.16	0.17	0.20
Total	100.12	99.81	100.06	99.99	100.05	99.78	99.85	100.10

*Analyst, R. Evans

the axis near 95°W and on the 85°W fracture zone, but are not considered here.

Petrographically, the basalts studied for RE show quenched textures typical of pillow basalts, and range from aphyric to plagioclase or, more rarely, olivine, porphyritic basalts. The new De Steiguer samples are classified as tholeiitic basalts according to Yoder and Tilley's[20] normative scheme (Fig. 3). They range from olivine normative to mildly quartz normative; and follow a Skaergaard trend on the AFM diagram (Fig. 3), with extreme iron enrichment reaching 18% FeO* for station D1, near 85°30′W. Cocotow rocks (J. W. Hawkins, unpublished), Southtow basalts[17] and RC10-56[19] fall on the same iron fractionation trend.

Basalts from stations Southtow D-17 and Batlett T6 tend to be alkali rich relative to common mid-ocean ridge basalts[21]. Since Fe_2O_3/FeO proportions were not determined by Anderson, *et al.*[17], Southtow D-7 and HFD-1 are not directly comparable with the De Steiguer samples. Anderson, *et al.*'s basalts[17] are, however, close to the Di normative join if Fe_2O_3 is assumed to be 1.5% (ref. 18). It is possible that the high alkali content of station T6 is attributable to alteration by sea water, and is not an intrinsic characteristic of melts erupted near the Hess Depression. On the other hand, the rare earth patterns of station T6 samples are depleted in light RE, and are typical of other tholeiitic basalts erupted along normal ridge segments (Fig. 4).

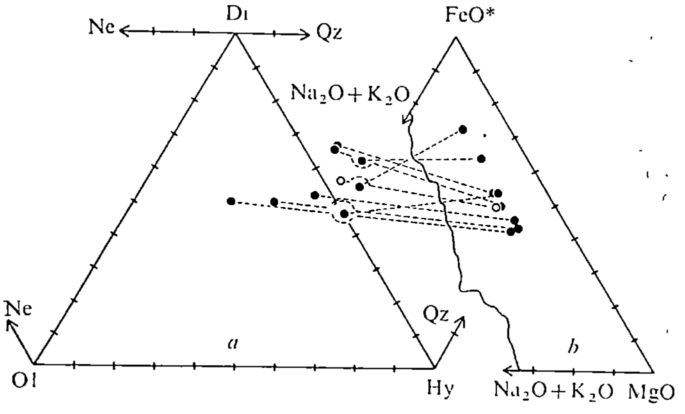


Fig. 3 *a*, Quartz – diopside – hypersthene – olivine – nepheline normative diagram of De Steiguer-dredged basalts: solid circles, light RE-depleted; open circles, RC-10-56 light RE-enriched basalt. No relationship is apparent between RE patterns and normative mineralogy. *b*, FeO* (total iron as FeO), Na_2O+K_2O, MgO diagram for the same samples Bulk chemistry data for De Steiguer samples are given in Table 2, and for RC-10-56 in ref. 19. Relationship between Fe/Mg variation and normative mineralogy is indicated by tie lines between the two diagrams.

RE variations along the ridge

Figures 2 and 4 show that all basalts located beyond 450 km west of east of the Galapagos Fracture Zone are depleted in light RE elements, including those from station T6 near the triple junction. Judging from such evidence[10], these basalts seem to have been derived from the light RE-depleted asthenosphere source, whether they were erupted within or outside the high magnetic amplitude region.

In contrast, ridge basalts enriched in light RE elements are found only near and just north of the Galapagos Archipelago (Figs 1 and 4). Some 190 km east of 91°W, an intermediate flat RE pattern is found. Yet, normatively, basalts from these later three stations are indistinguishable from the light RE-depleted basalts found further out from the Galapagos Islands (Fig. 3). Tholeiitic basalts with such light RE enrichment suggest mantle plume derivation[4,6]. The degree of light RE enrichment in these three stations is, however, too low relative to tholeiitic basalts from the Galapagos Archipelago to be 100% derived from the mantle plume[12,13].

It seems that the distribution of RE patterns along the ridge show no simple relationship with magnetic anomaly amplitudes. Basalts with both light RE-enriched and light RE-depleted patterns occur within the high magnetic amplitude region; and light RE-depleted patterns occur both within and outside the high magnetic zone as well. Judging from the RE patterns so far available, mantle plume mixing with DLVL material seems to be limited to a radius of approximately 450 km from the centre of the Galapagos Archipelago, and not to the entire length of the high magnetic amplitude zone.

Chemical correlations along the ridge axis

The variations of iron, titanium and light RE fractionation in basalts erupted along the Galapagos spreading centre are shown in Fig. 2 in relation to variations of the magnetic anomaly intensity, and ridge elevation. The chemical variations are compared with averages obtained along the East Pacific Rise (EPR) between 0 and 20°S. This part of the EPR seems geochemically typical of normal mid-ocean ridge segments[10,22], and it is regionally close to the Galapagos region. Figure 2 shows that outside the high magnetic anomaly region of the Galapagos spreading centre, the Fe content of the basalts closely straddles the average value found for the EPR; with the exception of somewhat lower values near 83°W. On the other hand, within the magnetic anomaly zone, there is a tendency for the Fe content to be somewhat higher than the EPR average; whereas the TiO_2 content is not greatly different from that in samples from outside the zone, and is generally close to that of the EPR. Finally, between 85 and 87°W unusually large FeO* and TiO_2 enrichments occur corresponding to the area of highest magnetic anomaly intensities. A relationship between magnetic intensity and Fe enrichment thus seems valid; but so far no obvious relationship is apparent with Ti outside the 85–87°W region. Further, it needs to be noted that low FeO* and TiO_2 values occur together with the high values in the area between 85 and 87°W. There also seems to be no systematic variation in Fe and Ti enrichment with distance or age across the ridge in the 85–87°W area.

Basalts from De Steiguer station D1 located on the ridge axis (~0 Myr), are the most Fe enriched (18% FeO*). Basalts from station 3D, dredged between magnetic anomalies 1 and 2 (~ 1.3 Myr), are close to the EPR average (9.2% ± 0.5 FeO*), and are thus normal. Basalts from station 4D, the northernmost station (~1.7 Myr), have intermediate FeO* contents (~15% FeO*). Both stations 4D and 3D were near the edge of the high amplitude zone. These observations suggest that the correlation between magnetic intensity and Fe–Ti content is not simple, as pointed out by Watkins (and Vogt and Johnson)[23]. Among other things, one would also have to know the relative volumes of iron-rich to normal lavas, to establish further a telechemical relationship between Fe (and perhaps Ti) enrichments and magnetic anomaly intensity.

The variation of the La/Sm ratio along the ridge shows a progressive decrease with distance (or depth) from the Galapagos platform; however, the ratio does not correlate directly with FeO* or TiO_2 content (Fig. 2). Thus, Vogt and Johnson's[16] idea of correlating Fe and Ti enrichments with $[La/Sm]_{EF}$ for the Galapagos spreading centre (and the Iceland–Reykjanes Ridge as well; J.-G. S., unpublished), seems to be invalid. The reason is that FeO*, TiO_2 and large ionic lithophile (LIL) element concentrations and their ratios in basalts, are not only functions of the type of mineral fractionation involved during partial melting in the mantle, fractional crystallisation during magma ascent, or stagnation in magma chambers, but also of the content of the mantle source(s) from which basalts are derived. No simple relationship can theoretically be predicted which would be of general application and independent of the geological processes and mantle sources involved. That should not, however, detract from the fact that the La/Sm ratio remains a reliable source indicator when dealing with tholeiitic basalts. Schilling[10] has shown that the La/Sm ratio of a tholeiitic basalt produced by some 20% or greater degree of partial melting, is practically identical to that of its mantle source, to within 20% or less. The ratio varies by more than a factor of two along the Galapagos spreading centre, and is independent of the Fe or Ti contents. The apparent decrease in the La/Sm ratio outwards from the Galapagos, suggests mantle plume mixing with DLVL material within a radius of approximately 450 km from the centre of the Archipelago. Even extensive shallow-depth fractional crystallisation of the olivine-gabbro type[24] cannot produce the light RE enrichment found in tholeiites occurring near the Galapagos Islands.

Fe enrichments of DLVL-derived basalts between 85 and 87°W

Figure 4 shows that the extent of RE enrichment and Eu depletion in basalts erupted between 85 and 87°W correlate closely with the Fe content of Galapagos lavas, although the degree of light RE fractionation (that is, the La/Sm ratio) remains rather insensitive to the Fe enrichment mechanism. We have modelled quantitatively such Fe and RE enrichments by fractional crystallisation. First, we have tried to satisfy the major element chemistry of the basalts using Wright and Doherty's[25] linear programming technique which allows determination of the type and extent of mineral extract. We then tested such mineral extracts against RE patterns of these rocks using the Doerner–Hoskin's logarithmic law for describing the change of the RE concentrations during fractional crystallisation[26]. In this process we found, as did Kay, *et al.*[18], that the Fe enrichment trend is accompanied by a decrease in the MgO, CaO and Al_2O_3 contents, and an increase in the TiO_2, P_2O_5 and, to a lesser extent, Na_2O contents; whereas the SiO_2 content stays practically constant, or decreases slightly. Contrary to the findings of Kay, *et al.*, however, we were not able to minimise SiO_2 residuals by extracting only olivine and plagioclase, but had also to extract a significant amount of clinopyroxene. Table 3 illustrates our point for an extreme case. This model shows that the most differentiated Fe-rich basalt, De Steiguer-D1, can be derived from a melt of the composition averaging Southtow D-7 by 75% fractional

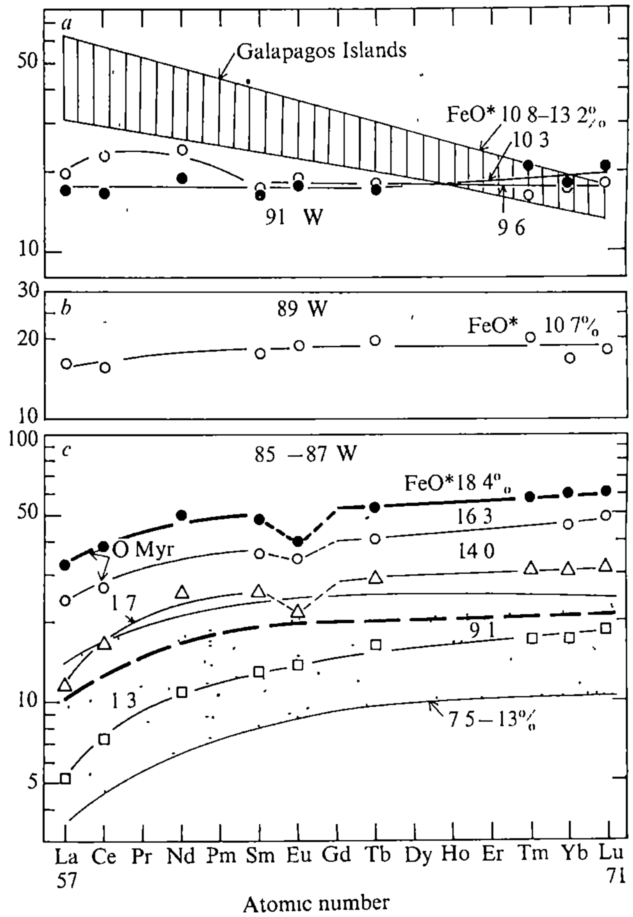


Fig. 4 Chondrite normalised rare earth patterns of dredged basalts from the Galapagos spreading centre. *a* and *b*, Basalts located within a radius of 450 km from the archipelego Hatched area, the field occupied by four Galapagos Island tholeiites studies petrochemically by McBirney and Williams[12]. Isabela (E-132), Baltra (E-28), Marchena (E-15) and the north-westernmost island of the archipelago, Darwin Island (E-35); $[La/Sm]_{EF}$ ratios are 1.67, 1.45, 1.15 and 1.49; and FeO*, 12.7, 13.2, 11.4 and 10.8 w%, respectively). Rare earth data from this laboratory (unpublished). *c*, Shaded area shows range obtained for basalts located outside a 450-km radius, east of 87°30'W and west of 95°W; all light RE depleted (see data in Table 1). De Steiguer stations located near the eastern end of the high magnetic amplitude region shown separately in relation to their FeO* content and their estimated magnetic age: D-1A (solid circle); D-1B (open circle); D-4 (triangle); average of D-3A and B (square). Note good correlation between total RE content and FeO*. Heavy dashed line is calculated model RE pattern of original melt discussed in text. RE and FeO* data taken from Table 1.

crystallisation of a mixture composed of plagioclase (An 62), clinopyroxene (En 48.5, Fs 10, Wo 41.5) and olivine (Fo 55), in proportions 0.54, 0.37 and 0.09, respectively. The proportions are reasonably close to the ternary eutectic (0.435 An, 0.49 Di and 0.075 Fo) experimentally determined for the simplified basalt system diopside–forsterite–anorthite[27]; thus adding some credibility to the model.

By inverting the problem, the RE pattern of the 'original melt' was calculated from the DS-D-1 residual melt RE pattern, using the crystallisation model, and the average crystal-melt RE partition coefficients listed in Table 3. The calculated RE pattern of the 'original melt' is shown in Fig. 4. It is light RE depleted and no longer shows any Eu anomaly, as expected The level of overall RE pattern enrichment satisfactorily falls within the range occupied by other 'normal ridge basalts' so far studied along the Galapagos spreading centre.

Table 3 Fractional crystallisation model*

	Model extraction					
Oxide	Primary melt (St 7-D7)	Clinopyroxene	Plagioclase	Olivine	Differentiated melt (DS D1A)	Residuals
SiO_2	50.90	51.50	52 78	36.73	50.75	−0 07
Al_2O_3	16 38	4.09	30.20	0 00	12 07	−0.02
FeO	7.60	5.66	0.00	31.92	15.84	−0 02
MgO	8.04	17.28	0.00	31.37	4.54	0.00
CaO	13.28	20.75	12.57	0.00	9.38	−0 01
Na_2O	2 50	0.22	4.47	0.00	2.79	0.27
K_2O	0.09	0.00	0 00	0.00	0.26	−0.07
TiO_2	1 04	0.36	0.00	0 00	3.80	0.02
P_2O_5	0.07	0.00	0.00	0.00	0.34	0.09
MnO	0 16	0 19	0.00	0.00	0.28	−0 14

Total mineral extract: 75%.
Mineral extracts: 28% Cpx.; 40 5% Plag., 6.5% 0l.
Modal mineral extracts: 0.37 Cpx.; 0.54 Plag.; 0.09 0l.
Plag. = An 62. Oliv. = Fo 55. Cpx. = Wo 41.5; En 48 5; Fs 10.0

Rare earth partition coefficients†			
Element	K_{cpx}	K_{plag}	K_{ol}
La	0.180	0 172	0.0089
Ce	0 256	0 153	0.0090
Sm	0.571	0.092	0.0105
Eu	0 610	0.522	0.0110
Yb	0.560	0 0781	0.0230
Lu	0.540	0.0810	0.0270

*Minimum residuals were obtained by arbitrarily disregarding Fe_2O_3 contents and recalculating the analyses to 100% on a water free basis
†K is defined as trace element concentration in crystal relative to the melt.

We conclude that the shallow-depth fractional crystallisation model satisfactorily explains the Fe-rich basalts and associated RE patterns occurring near 86°W. The shallow-depth crystallisation process seems to be dominated by extractions of plagioclase, and to a lesser extent of pyroxene and olivine, from melts originally derived from the DLVL source. The model is thus consistent with Anderson, *et al.*'s[17] suggestion, but seems to be limited to the 85–87°W area.

Finally, we emphasise that even in the extreme case of some 18% FeO* enrichment apparently requiring some 70–80% extent of crystallisation, the RE patterns of such residual liquids have retained the light RE-depleted patterns of the original melt, characteristic of normal ridge segments, although they develop a noticeable Eu depletion relative to RE neighbours, and a higher overall level of enrichment. In contrast to the 85–87°W area, ferrobasalts[12] from James Island (FeO* = 12 4%) and Jervis Island (FeO* = 13.4%) are light RE enriched (La/Sm_{EF} = 1 53 and 2.03, respectively) and are comparable with other subaerial tholeiites from the archipelago (Fig. 4). Those considerations suggest that the light RE-enriched patterns found in the two basalts erupted along the Galapagos spreading centre just north of the archipelago, can hardly be the product of shallow-depth fractional crystallisation. The two basalts show no Eu depletion or overall enrichment of the relative RE patterns, nor do they show any FeO* enrichments. More likely, these later basalts were derived from a mantle source significantly enriched in light RE elements relative to the DLVL-source feeding normal segments of the Galapagos spreading centre.

Conclusions

Several points need to be emphasised on the basis of this pilot study.

- Both light RE-enriched and depleted types of RE patterns occur within the region of high magnetic anomaly amplitudes. Light RE-depleted patterns also occur both within and outside the zone of high magnetic anomaly amplitudes. Thus, no simple relationship is readily apparent between types of RE pattern and the intensity of magnetic anomaly amplitudes.
- The highest Fe and Ti enrichments occur where the magnetic anomaly amplitudes are also largest (between 85 and 87°W) The RE and major elements in these basalts suggest unusually extensive, shallow-depth fractional crystallisation of the olivine gabbro type occasionally found along normal ridge segments[10,18,22]. The crystallisation model based on the new De Steiguer samples is thus consistent with Anderson, *et al* 's[17] explanation for the high magnetic anomaly amplitude near 86°W.
- On the other hand, our preliminary data suggest that a shallow-depth olivine gabbro crystallisation mechanism, such as that found between 85 and 87°W, is apparently not the mechanism responsible for the high magnetic anomaly across the whole region, contrary to the suggestion of Anderson, *et al.*[17] (see Fig. 2). That is evident from the fact that no overall RE enrichments and relative Eu depletions are found within this zone, nor is the Fe content of the basalts markedly and systematically higher, except between 85 and 87°W. Additional sampling is required to resolve the question of the high magnetic amplitude region. Mild Fe enrichments in the area cannot be ruled out as an explanation.
- The telechemical idea[16] of correlating magnetic anomaly amplitudes directly to Fe and Ti content of basalts, remains a complex and open question. Similarly, the idea[16] of using the intensity of magnetic amplitudes to infer Fe and Ti contents, and in turn an array of LIL elements (and by inference their ratios as well) and Pb and Sr radiogenic isotopes, has no general validity, since Fe and Ti enrichment may have several causes. For example, Fe and Ti enrich-

ments can be obtained from either a mantle depleted in light RE along normal ridge segments (for example, as at the Galapagos spreading centre near 86°W), or from a mantle with light RE-enriched patterns and relatively richer in Sr and Pb radiogenic isotopes (for example, in the mantle beneath Iceland and the northern part of the Reykjanes Ridge[4,7,9], and the Galapagos Islands[12]). Thus, the idea[16] of using magnetic amplitudes to infer Fe and Ti contents to identify plume-derived oceanic crust, is ambiguous on this basis alone, and could be misleading.

- Basalts with light RE-depleted RE patterns, characterising 'normal ridge segments' are confined to east of 87°W, and west of 95°W. On this basis, the two segments of the Galapagos spreading centre situated between 95°W and 101°W (including the triple junction with the East Pacific Rise) and 87° to at least 83°W, are considered 'normal ridge segments'. Basalts erupted along these two segments seem to have been derived from the DLVL mantle source.
- So far, ridge basalts with RE patterns similar to those of light RE-enriched mantle plumes are confined to a radius 450 km from the centre of the Galapagos Archipelago. Maximum $[\text{La/Sm}]_{\text{EF}}$ values (1.1) are found just north of the main islands, but these values seem to be diminished already by mixing with DLVL material, relative to tholeiitic basalts from the Galapagos Islands[12,13]. The data are consistent with the presence of a mantle plume rising beneath the Galapagos platform. The sampling along the Galapagos spreading centre, however, is so far insufficient to decide whether the mantle plume flow in the asthenosphere occurs radially about the Galapagos platform; or preferentially along certain directions, such as generally northward towards the Galapagos spreading centre, or perhaps channelled along the major Galapagos Fracture Zone. Our data are also insufficient to infer whether the Galapagos mantle plume is, or is not, richer in Fe and Ti contents relative to the surrounding DLVL mantle source.
- Finally, the cause of unusually extensive shallow-depth fractional crystallisation of DLVL-derived basalts near 86°W, remains puzzling. Unusually extensive hydrothermal activity has been reported in the area[29,30] compared with elsewhere on the Galapagos spreading centre[31]. It is conceivable that unusually intense hydrothermal circulation might have been removing heat more efficiently from a magma chamber generated by spreading, thus enhancing locally fractional crystallisation before lava eruption on the sea floor. The model remains to be quantified in terms of the various rates involved.

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letters to nature

Transient X-ray source A0620–00 observed at 408 MHz

THE transient X-ray source A0620–00 reported by Elvis, *et al.*[1] was also detected as a transient radio source at a number of observatories[2-5] at frequencies from 962 to 5,000 MHz. We report here the detection of the source at 408 MHz using the Molonglo radio telescope on August 20, 1975. Since the result is based on a single observation we shall discuss the steps taken to ensure that the detection is real.

The Molonglo telescope is a transit instrument with 11 simultaneous beams in declination covering a strip of sky 15′ wide. Because of the large uncertainty in the position of the source available to us at the time, several nights were required to map the area. By the time we became aware of a reliable position for the source it was no longer detectable above the noise level.

A source of 0.3 Jy was observed on August 20, very close to the accurate position of the object identified with the X-ray source[6]. No such source was detected on a previous survey record nor on records taken subsequent to the decay of the X-ray source. As the r.m.s. noise for a single observation is 0.04 Jy, the detection level of ~7 s.d. is well above the possibility of a random noise fluctuation. The 408-MHz position compared with that for the optical source is

optical	06 h 20 min 11.2 s	−00°19′10″
408 MHz	06 h 20 min 11.6±0.6 s	−00°18′18±20″

The right ascension agrees extremely well but the declination differs by 2.6 s.d. The probability of a radial discrepancy of this order is ~3%, so it is not highly significant on the assumption of noise errors alone.

Table 1 Flux density

Date	Flux density at the optical position (Jy)
December 29, 1973	−0.08
August 20, 1975	+0.28
September 6, 1975	+0.02
September 7, 1975	0.00
September 17, 1975	−0.03
December 28, 1975	−0.08

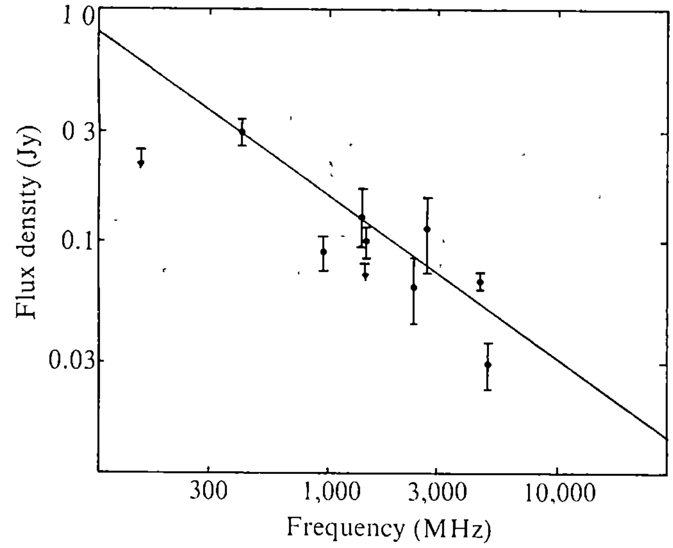

Fig. 1 The flux density of the radio source associated with the X-ray object A0620-00 plotted as a function of frequency. All points have been reduced to the common date of August 19.9, 1975 (UT). A line corresponding to a non-thermal spectrum (see text) is shown for comparison.

There remains the possibility of confusion with a weaker source or with a weak side lobe which may be either positive or negative, These effects are normally very small but in the galactic plane cannot be entirely ignored. We have therefore calculated the flux density at the optical position for all days on which this region of sky was observed (Table 1).

The estimated r.m.s. noise error on each of the above measurements is 0.04 Jy. The mean of all days other than August 20, 1975 is −0.03±0.02 Jy. The relative flux density on August 20 is 0.31±0.05 Jy which is highly significant and free of confusion. Based on the rate of decay of the source at higher frequencies the expected flux density at 408 MHz on September 6–7 would be <0.01 Jy and these have been considered as background points.

The flux density at 408 MHz is much larger than the higher frequency values. The available data are plotted in Fig. 1. The points have been reduced to the common epoch of August 19.9 UT using decay rates where available and using a time constant of 4.5 d for all other data.

The high flux density at 408 MHz is consistent with the suggestion of Owen *et al.*[7] that the spectrum could be non-thermal. In fact a spectral index α ($S = S_0\nu^{\alpha}$) of ~ -0.7 as shown in Fig. 1 fits the data quite well except that the points obtained with 3″ resolution tend to fall somewhat below the line.

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Explanation for the very low Ga and Ge concentrations in some iron meteorite groups

THE Ge/Ni ratios in iron meteorites range from 10^{-2} to 10^{-7} atoms/atom (ref. 1). Gallium is strongly correlated with Ge, but the total range in Ga/Ni ratio is only about 2,000. Iron meteorites tend to have Ga and Ge concentrations within various restricted ranges—thus their usefulness for the classification of iron meteorites. No other element correlates strongly with Ga and Ge. Here we point out parallels between the abundance patterns of moderately volatile elements in iron meteorites and ordinary chondrites, and discuss how these can be related to condensation processes in the solar nebula.

Most (83%) of the irons have Ge/Ni ratios in the range $(0.2–6.3)\times10^{-3}$ atoms/atom, in the same range as ratios in primitive, volatile-rich CI chondrites, 2.6×10^{-3} atoms/atom and in other groups of chondritic meteorites[1,2]. Two iron meteorite groups show much lower Ge/Ni ratios. $(1.0–1.2)\times10^{-6}$ atoms/atom in Group IVA and $(1.6–2.9)\times10^{-7}$ in Group IVB. The processes responsible for these very low Ge concentrations were clearly much more effective than those that affected the chondritic meteorites.

Concentrations of Ga and Ge in iron meteorites were probably established either during condensation[3] at the nebular location where that parent body formed and/or during processes occurring within each parent body.

Wai *et al.*[4,5] showed that core formation in a planetary body does not result in fractionation of Ge from Ni. By default, it seems that the fractionation of Ge and Ga from Ni must have occurred during the condensation and agglomeration of the solar nebula

Gallium and germanium belong to a group of moderately volatile elements having abundances 1.2–10 times lower in ordinary chondrites than in CI chondrites, which are generally believed to be most representative of mean Solar System abundances[6]. It is instructive to compare Ga and Ge with two other moderately volatile elements, As and Cu. In Fig. 1 data for Group IVA and IVB irons are compared with IIIAB irons and H-group ordinary chondrites; element/Ni ratios are normalised to CI chondrites

The H/CI ratios in Fig 1 show a steady decrease from As to Ge; Wasson and Chou[6] suggested that nebular condensation temperatures decrease similarly, and that a nebular mechanism existed which resulted in increasing loss of gas with decreasing condensation temperature. Wasson and Chou discussed several nebular processes which could result in such loss patterns.

Figure 2 shows equilibrium condensation activities of the most stable compounds of Cu, Ga and Ge at nebular pressures of 10^{-4} and 10^{-6} atm. No calculations are reported for As, since data could not be found for a number of potentially important compounds. Equilibrium between gases and solids and ideal solution was assumed. Mean Solar System abundances of C, H and O were taken from Cameron[7], and for Cu, Ga and Ge from Wasson and Chou[6]. Free energy data were taken from the JANAF (1971) tables for CO, H_2O and Cu compounds[8], and from other recent sources for Ga (refs 9 and 10) and Ge (refs 11–14).

In a departure from earlier calculations regarding trace element condensation[15,16], the ordinate does not show the fraction of the element removed from the gas phase, but rather the equilibrium activity in each phase divided by that expected if the element condensed entirely in this phase. This representation allows more insight into the siting of each element during equilibrium processes in the nebula. For the six phases of importance these normalising CI ratios were· Cu metal, $Cu/(Fe+Ni)=6.1\times10^{-4}$; $CuS_{0.5}$,

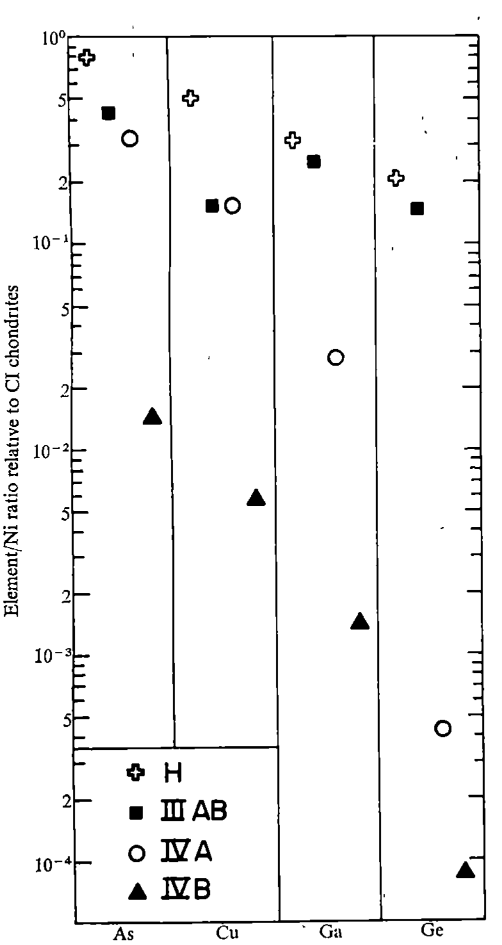

Fig. 1 CI chondrite normalised element/Ni ratios of four moderately volatile elements are generally similar in H-group chondrites and IIIAB irons. The IVB irons have very low ratios of each volatile, whereas the IVA irons have normal amounts of two volatiles (As and Cu) and very low amounts of two others (Ga and Ge).

unity above 680 K, Cu/S=5.4×10^{-3} below 680 K; Ga metal, Ga/(Fe+Ni)=4.3×10^{-5}; $GaO_{1.5}$, Ga/Al=4.8×10^{-4}; Ge metal, Ge/(Fe+Ni)=1.3×10^{-4}; Mg_2GeO_4, Ge/Si=1.2×10^{-4}. An implicit assumption of this treatment is that unit activity of a trace element results from complete substitution for the element in the denominator (the exception is Cu for Fe in FeS).

Scott[17] noted that the IVB irons are enriched in refractories (Re, Os, Ir) and depleted in volatiles and concluded that they represent high temperature nebular condensates. The data in Fig. 1 cannot be explained by equilibrium condensation processes, however. This conclusion is independent of pressure, but can be conveniently demonstrated by consideration of the condensation curves at a pressure of 10^{-4} atm (Fig. 2*a*). In order that only 0.6% Cu condenses, equilibration with nebular gases must cease at a temperature of about 1,350 K. At this temperature, however, the equilibrium Ga and Ge activity ratios are 2.2×10^{-4} and 5.3×10^{-5}, substantially lower than IVB values. Kelly and Larimer[3] reached a similar conclusion.

The moderately volatile element pattern in IVB is in fact quite similar to that in ordinary chondrites (Fig. 1) The difference is one of degree, implying that the same loss mechanisms were responsible but that the IVB process was much more efficient.

A plausible picture is the following. Condensation at the Group IVB location occurred during a period when the temperature decreased rapidly. Diffusion was too slow to allow trace metals to reach the centres of Fe–Ni grains, and solution occurred only in the outermost atomic layers. As temperatures continued to fall, trace metals condensed as a fine aerosol that was later blown away by a T-Tauri solar storm. The IVB irons have high Ni contents suggesting that an appreciable amount of Ni-poor metal was lost, possibly by the same mechanism. Condensation at the H-group location was more complete because the temperature decreased less rapidly, and trace metals could diffuse deeper into Fe–Ni grains.

As noted by Scott[17] the abundances of As and Cu in Group IVA irons are nearly identical to those in Group IIIAB, whereas Ga and Ge are lower in IVA by factors of about 10 and 300, respectively. The IIIAB abundances are nearly identical to those in the H group, and it is likely that the same process was responsible for the loss of moderately volatile elements at both nebula locations.

At the IVA location conditions were also similar as the nebula cooled to the condensation temperature of Cu, but below this temperature condensation was much less complete. We can think of two ways in which IVA conditions may have differed from IIIAB or H conditions: either (1) the rate of temperature decrease increased abruptly below the Cu condensation temperature, and incomplete condensation and loss of Ga and Ge occurred in conditions similar to those proposed for the loss of volatiles at the IVB location; or (2) early condensation occurred in equilibrium conditions, but below some limiting temperature the gas and solids became physically separated and no further condensation was possible[3]. If the second mechanism is viable, we should find temperatures in Fig. 2 which account for the Ga and Ge depletions in IVA relative to those in Group IIIAB. At a pressure of 10^{-4} atm 1,060 K could provide the correct factors. At 10^{-6} atm no temperature exactly matches the IVA/IIIAB depletions; the best fits are obtained at about 820 and 900 K. This double value results from the tendency of Ga preferentially to enter the silicates below 860 K. The temperature of equilibration with nebular gases cannot have been less than 820 K, since lower temperatures result in sharp increases in the Ge/Ga ratio in Fe–Ni. This lower temperature is implausible, however, since the Ga concentration in the silicates is high, and this Ga should re-enter the metal at the high temperatures needed to bring about gravitational separation of metal from silicate in order to form a core

Determination of a siderophilic element (that is, one tending to remain in the metallic state) more volatile than Ge offers a means for choosing among the two IVA models. The model involving a change in cooling rate should result in higher concentrations of such an element than that incorporating a minimum equilibration temperature. Unfortunately, the same atomic forces that cause some elements to show siderophilic properties tend to produce moderately high nebular condensation temperatures. We know of no siderophilic element more volatile than Ge. The most siderophilic of the more volatile elements is Zn, which has a condensation temperature close to 680 K. Published data on Zn show considerable scatter, but it seems that metal/sulphide concentration ratios can be as high as 0.1. Smales *et al.*[18] reported Zn concentrations less than their detection limit of 1 μg g^{-1} in IVA irons. We are initiating a neutron activation study of Zn in these meteorites which will allow detection down to ng g^{-1} levels.

Although S has a pressure-independent condensation temperature (680 K, Fig. 2) lower than that of Ge at 10^{-4} atm, and the same as Ge at 10^{-6} atm, CI normalised S/Ni ratios in IVA and IVB irons are ~ 1×10^{-2} and 7×10^{-4}, respectively—factors of 8 and 20 higher than those of Ge. This difference may result in part from the differences in condensation reactions. Whereas Ge and Ga can condense as pure metals, S must form FeS by reaction with the surfaces of metal grains. The latter reaction should proceed rapidly until all grains are coated with

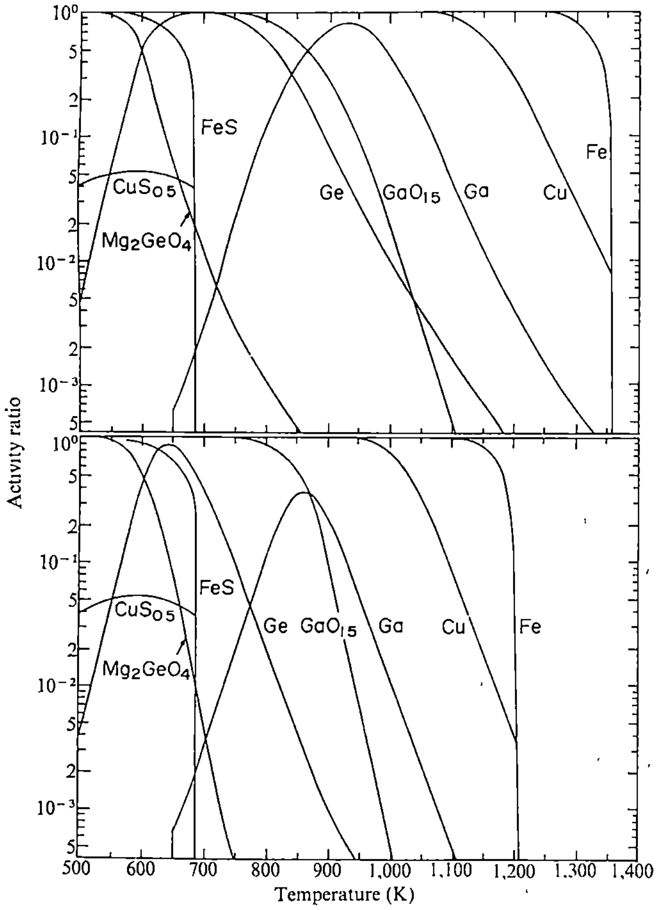

Fig. 2 Nebular condensation of Cu, Ga and Ge at pressures of 10^{-4} atm (*a*) and 10^{-6} atm (*b*); the ordinate gives the equilibrium activity of the substance divided by that expected from full condensation of the trace element in this chemical form. At high temperatures Ga and Ge condense as the metal but at low temperatures the oxide form becomes dominant. Some Cu enters FeS when the latter becomes a stable phase at 685 K.

FeS. At moderate temperatures (>500 K) there is no other reaction by which an appreciable fraction of the S can condense, thus it cannot undergo homogeneous condensation as a fine aerosol capable of being blown away by a T-Tauri wind.

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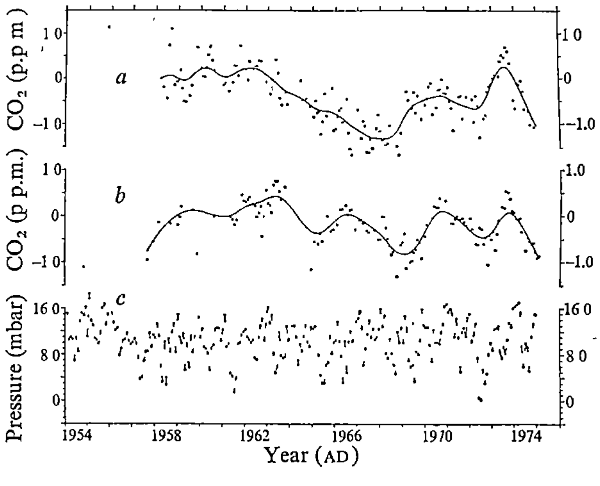
Modulation of atmospheric carbon dioxide by the Southern Oscillation

ATMOSPHERIC CO_2 records for the South Pole and Mauna Loa Observatory, Hawaii, show a seasonal variation, presumably arising from the uptake and release of CO_2 by vegetation, and a long term increase, almost certainly caused by combustion of fossil fuel. The increase is much greater in some years than in others[1,2]. Changes in the rate of fossil fuel combustion are not likely to be the cause of the variation in yearly increase, as combustion has increased very steadily[3]. I present here evidence that the variation is connected to the Southern Oscillation, a large scale atmospheric and hydrospheric fluctuation with an irregular period of 1–5 yr (ref. 4). The connection, if present, indicates that a principal cause of the variation may be a change in the rate of removal of CO_2 by the oceans.

The CO_2 records, approximately as they would appear if there were no seasonal effect and no long term increase, are shown in Fig. 1. These 'anomaly curves' were obtained by an iterative procedure in which the data were first fitted to a long term increase and a seasonal effect, represented, respectively, by a spline function[5] (with stiffness set by eye) and by an average over identical months, for each year, of the difference between the data and the spline. A constant airborne fraction multiplied by an integral of the fossil fuel consumption rate was then subtracted from the spline (long term increase) to remove the effect of fossil fuel combustion. The analysis will be discussed in more detail elsewhere.

The difference in average monthly barometric pressures between Easter Island and Darwin, Australia, is a suitable indicator[6] of the Southern Oscillation, and will be called the Southern Oscillation Index (SOI). Easter Island is near the centre of a subtropical high pressure cell, and Darwin is representative of the equatorial low pressure zone. The SOI is directly related to the strength of the easterly trade winds that blow along the Equator. When the SOI is high, the trade winds are stronger than average. The middle latitude westerlies along the southern flank of the subtropical

Fig. 1 Atmospheric CO_2 records at *a*, Mauna Loa, Hawaii and *b*, South Pole, with seasonal effect and fossil fuel increase removed by a procedure described in the text. *c*, Southern Oscillation Index. Data assembled by W. H. Quinn, Oregon State University.



anticyclone belt also tend to blow more strongly. The Humboldt Current and equatorial currents are accelerated, thereby increasing upwelling along the coast of South America and along the Equator. The equatorial countercurrent, which flows against the trade winds, is decelerated. Low SOI brings reduced trade winds and reduced middle-latitude westerlies. The equatorial countercurrent flows more strongly and carries warmer water to the eastern Pacific[7]. Low SOI is often accompanied by El Niño, an invasion of warm water off the west coast of northern South America, which brings disaster to the fishing industry and torrential rain to a normally arid region.

Correlation maxima[8] for the South Pole anomaly with the smoothed SOI (12-month moving average) near zero lag are 0.40 with a positive SOI lag of 3 months and −0.45 with a negative SOI lag of 17 months (Fig. 2). If these two time series were uncorrelated, the cross-correlation function would be expected to be zero, with a standard deviation, near zero lag, of approximately 0.29. The statistical confidence level for the existence of a connection is thus about 83%. The correlation maxima with positive SOI lag of three months would not seem to meet the requirement of a causal relationship, since one would expect the Southern Oscillation to be the cause of change in atmospheric CO_2 level, and not the reverse. (The possibility exists that the anomaly in CO_2 level drives the Southern Oscillation, but it seems unlikely because the CO_2 changes are only 1 part per million (p.p.m.) in a total of about 330 p.p.m.) The negative, 17-month SOI lag is physically possible, but rather long. Corresponding correlation maxima for the Mauna Loa anomaly are smaller: 0.14 with an SOI lag of 6 months, and −0.28 with a negative SOI lag of 10 months. The standard deviation, if the series were unconnected, would be 0.18 near zero lag. Consequently, none of these correlation maxima is entirely satisfactory.

An atmosphere and hydrospheric circulation phenomenon should directly affect the rate of change of the CO_2 level instead of the level itself. For example, the transfer of CO_2 across the air–sea interface is governed approximately by an equation of the form

$$-(\mathrm{d}p/\mathrm{d}t) = k(p-P) \qquad (1)$$

where p is the CO_2 partial pressure in the atmosphere, P is the CO_2 partial pressure in the ocean beneath a thin boundary layer, and k is a rate factor which depends strongly on the boundary layer thickness, and consequently on wind shear[9]. It is therefore reasonable to correlate the derivatives of the anomaly curves with the smoothed SOI (Fig. 3).

Correlation maxima are then −0.64 with a negative SOI lag of 6 months for the South Pole, and −0.55 with a negative SOI lag of 2.5 months for Mauna Loa. Standard deviations near zero lag would be 0.34 and 0.28, respectively, if the series were uncorrelated. Not only are the derivative anomaly correlations higher, relative to the standard deviations, but the time lags are more reasonable.

The nominal confidence level for the existence of a connection between the time derivatives of the anomalies and the Southern Oscillation is about 95%. The true confidence level, however, is probably smaller because of the extensive data manipulation and smoothing. Longer CO_2 records should resolve whether a connection exists.

The influence of the Southern Oscillation on the atmospheric CO_2 level seems to be felt 3.5 months earlier at Mauna Loa than at the South Pole. The reason for this may be that the South Pole is in a relatively fixed high pressure zone, isolated from the lower latitudes by circumpolar winds[10].

Increased loss of CO_2 from the atmosphere during periods of high SOI must be attributable to increased uptake by one of the two natural reservoirs with which atmospheric CO_2 principally exchanges: the oceans and the biota. If a high SOI were associated with increased rainfall over arid but otherwise fertile land, increased vegetative uptake could explain the correlations. Data to test this hypothesis are probably available, but have not been examined in the course of this study. The large areas of land that would have to be involved make this explanation seem unlikely, however.

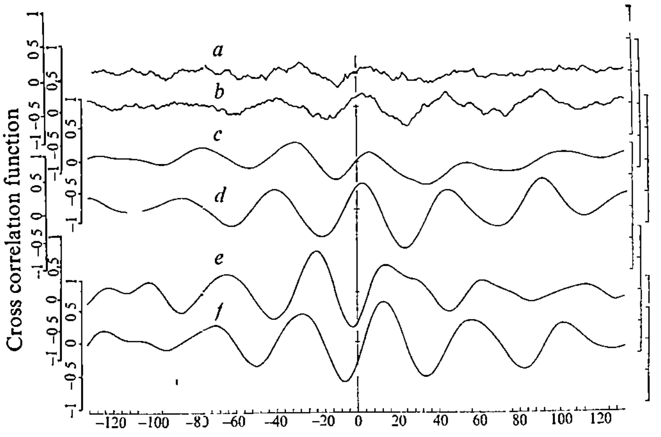
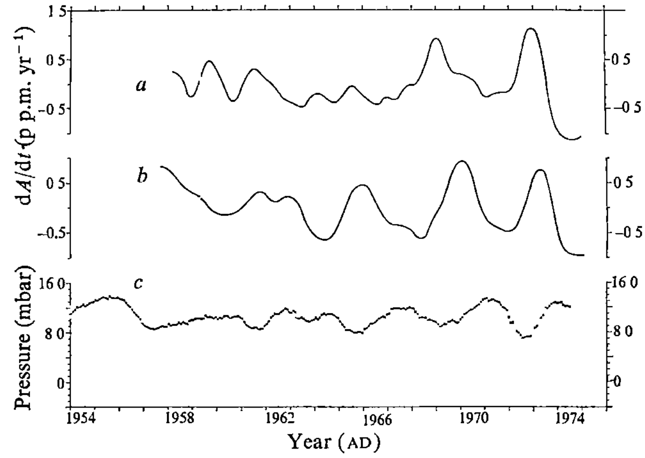


Fig. 2 Cross correlation functions for Mauna Loa and South Pole CO_2 records and the SOI: *a*, unsmoothed Mauna Loa anomaly data (with missing data filled in by interpolation) and unsmoothed SOI; *b*, unsmoothed South Pole anomaly data (with missing data filled in by interpolation), and unsmoothed SOI; *c*, Mauna Loa anomaly curve and smoothed (12-month moving average) SOI; *d*, South Pole anomaly curve and smoothed SOI; *e*, derivative Mauna Loa anomaly curve and smoothed SOI; *f*, derivative South Pole anomaly curve and smoothed SOI. All correlation functions are plotted against the lag of the SOI. Standard deviations, if each pair of curves were uncorrelated, would be (*a*–*f*): 0.09, 0.12, 0.18, 0.29, 0.28, and 0.34.

The oceans transfer CO_2 to the atmosphere in regions where there is upwelling: along the Equator and along the western margins of continents, and absorb CO_2 from the atmosphere in many other regions where the CO_2 partial pressure exerted by the oceans is less than that of the atmosphere[11].

The Southern Oscillation has global influence[12], and indeed may be a fluctuation in the intensity of the general atmospheric circulation. Kanwisher has shown that the CO_2 exchange rate (essentially the coefficient k in equation (1)) varies as the square of the wind speed[9], so a small increase

Fig. 3 Time derivative CO_2 records at *a*, Mauna Loa and *b*, South Pole, obtained by differentiating corresponding curves in Fig. 1. *c*, SOI, smoothed by a 12-month moving average, shown for comparison.



in average wind speed at high SOI could cause increased uptake by the oceans.

If the Southern Oscillation is mainly localised in the South Pacific, the explanation may be that increased ocean uptake occurs in the zone of westerly winds from ~ 40–50° S. This region should have a very high exchange rate for CO_2 because of the strong winds and large ocean area[9], and the westerly winds in this zone correlate with the easterly trade winds to the north[10], and thus with the SOI.

On the other hand, a high SOI should also bring increased upwelling, at least along the western coast of South America and along the equatorial Pacific. The observed correlations seem to indicate that the stronger absorption by the oceans dominates the effect of the increased upwelling.

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D-region rocket measurements in winter anomaly absorption conditions

The winter anomaly in ionospheric absorption was first identified by Appleton[1], and although extensively studied over the past 40 yr it still remains for the most part a major unsolved problem. Its principal feature is that at latitudes above ~35°, on most winter days, h.f. ionospheric absorption is large (often larger than on summer days at 1200). Furthermore, there is considerably greater variability in the day-to-day absorption in winter than in summer, and the magnitude of the anomaly seems to increase with latitude, although at high latitudes the phenomenon is largely masked by other disturbances characteristic of the auroral zone. Rocket measurements have shown that on winter anomaly days there is a considerable enhancement of the electron density of the lower ionosphere particularly in the height range 80–90 km (refs 2–4). Calculations indicate that these enhanced electron densities would be sufficient to account for the winter anomaly absorption without involving any changes in other parameters which determine the magnitude of h.f. absorption such as the collisional frequency ν.

On a recent coordinated firing of three Petrel payloads from South Uist (57.4° N, 7.4° W) at approximately 1200 on November 29, 1974 we measured ion and electron concentrations on a day of very marked winter anomaly absorption. The first rocket was instrumented by the Max-Planck-Institut, Heidelberg and carried a quadrupole mass spectrometer to measure positive ions and neutral constituents. The second rocket was instrumented by the Appleton Laboratory to measure atomic oxygen and also carried a Langmuir probe to measure relative values of the electron concentration. The third rocket was instrumented by the University College of Wales, Aberystwyth and carried a Faraday rotation radio experiment on two frequencies (which provided absolute measurements of the electron concentration) and also carried a Langmuir probe identical to that flown on the second rocket. Mass spectrometer measurements of positive ion composition were obtained over a height range of 70–115 km and it was observed that above 72 km NO^+ was by far the most abundant species. This is in contrast to the normal situation in which O_2^+ ions reach abundances comparable with NO^+ above about 85 km, and water cluster ions are dominant below that height[5]. Relative measures of electron density by the Langmuir probes were obtained from 60 to 130 km and absolute values by the Faraday rotation technique were obtained from 70 to 90 km. These various results are shown in Fig. 1. For comparison purposes Fig. 1 also shows electron density curves for about the same solar zenith angle but measured (1) on a winter day of below average absorption and (2) on a summer day.

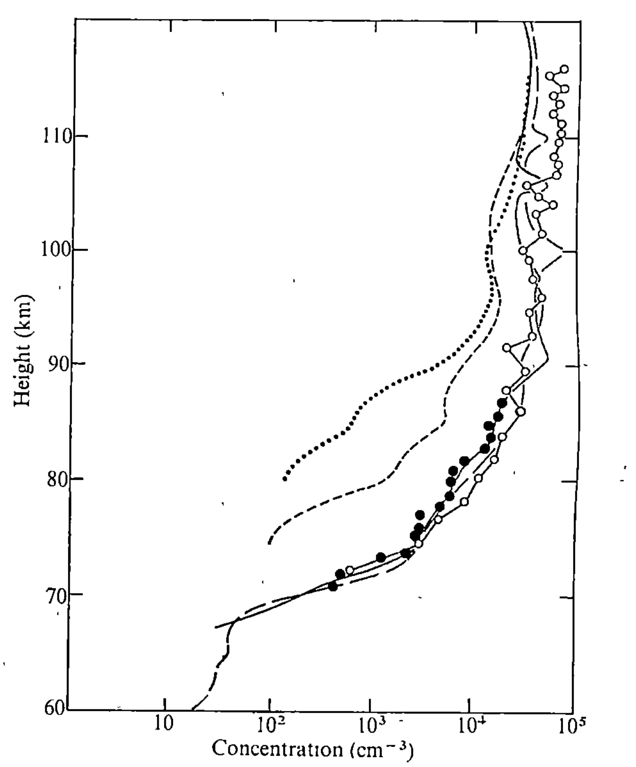

Fig. 1 Comparison of NO^+ ion and electron concentrations (Ne) during severe winter anomaly absorption at South Uist, Scotland (57.20° N, 7.14° W). Also shown are electron concentrations for normal winter and summer conditions at similar solar zenith angles χ. November 29, 1974—winter anomaly day, $\chi \approx 80°$: o—o—o, NO^+ flight, P182H, 1117 UT; ————, Ne (probe), P175H, 1153 UT; ————, Ne (probe), P179H, 1309 UT; • • •, Ne (Faraday rotation) P179H. December 6, 1971, normal winter day, $\chi \approx 83°$: – – – – – –, Ne (Faraday rotation + probe), P83H, 1402 UT. June 26, 1971, summer day, $\chi \approx 80°$. ······, Ne (Faraday rotation + probe), P120H, 1944 UT.

It can be seen that throughout the height range 70–115 km the profile of enhanced electron density on the winter anomaly day is practically coincident with the profile of NO^+ concentration, a result which points strongly to the conclusion that winter anomaly absorption occurs as a result of a greatly enhanced concentration of NO particularly at D-region heights.

Earlier work on the spatial and temporal morphology of the winter anomaly absorption phenomenon has indicated that its incidence may well be associated with the occurrence of some fairly large scale atmospheric movements in the lower ionosphere. If further experimental results support the conclusion indicated by the present rocket results concerning the role of NO, and if further progress with the problem is to be made, an explanation will be needed for the enhanced production of NO and/or its transport to the locations concerned during the winter months.

A fuller account of this work and of other results obtained in these rocket flights will be published elsewhere.

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Recent activity of Stromboli

DURING a recent extensive phase[1] on Stromboli (November 5–24, 1975; Fig. 1), which was accompanied by an increase of explosive activity, I noticed an interesting inverse relationship between seismic and eruptive activity.

The recent extensive phase was the first since 1971. In October 1972 the crater terrace was an oval shaped basin with five vents, aligned along a NE–SW fissure (Fig. 2*a*); four of the vents were active. In December 1972 two strong explosions occurred, and large fragments of old lava fell near San Bartolo. In April 1974 a fissure appeared in the central part of the 'Fossa'; three cones were built by the explosive activity along this fissure between May and August (Fig. 2*c*). On September 19, 1974 at 0010 GMT two big explosions occurred; after the explosions I observed that the cinder cones had been destroyed and that an irregular elongated crater with three vents had taken their place (Fig. 2*d*).

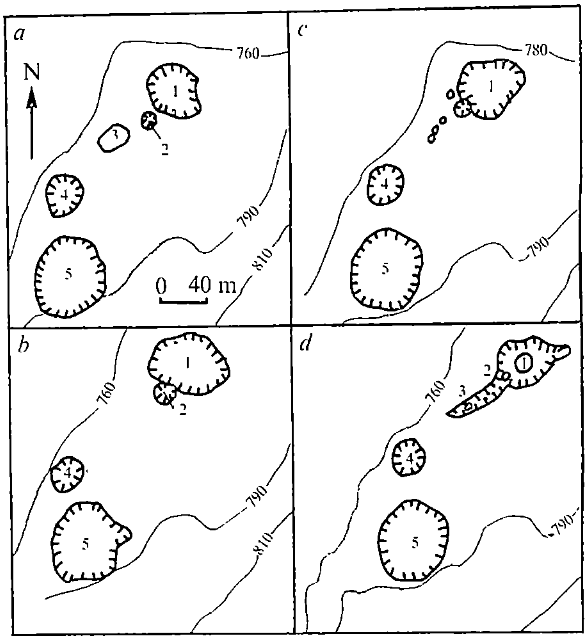
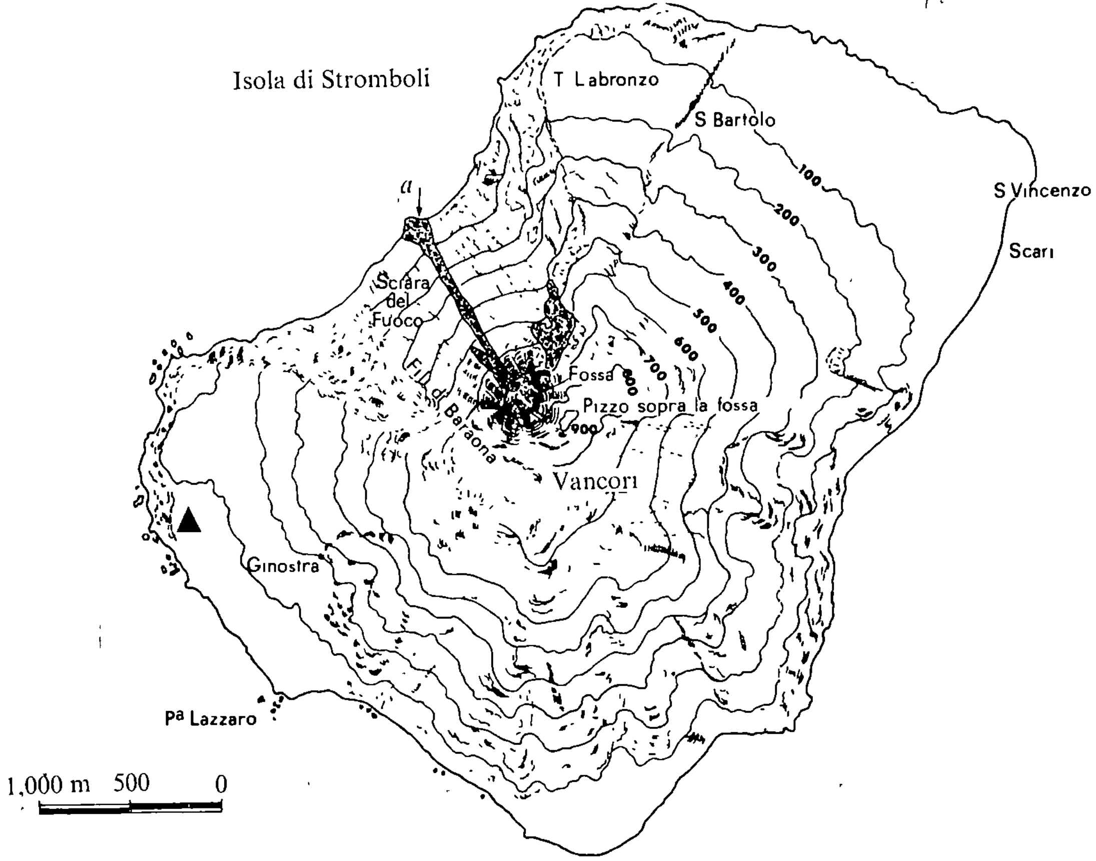


Fig. 2 Evolution of the crater plain (Fossa): *a*, October 1972; *b*, May 1973; *c*, May 1974; *d*, December 1974. Since the explosions of September 1974 vents 1, 2, 3 are all inside an elongated crater (*d*).

Fig. 1 Sketch map of Stromboli: the two most recent lava flows, the seismic station of Ginostra (▲), and 1975 lava flow.



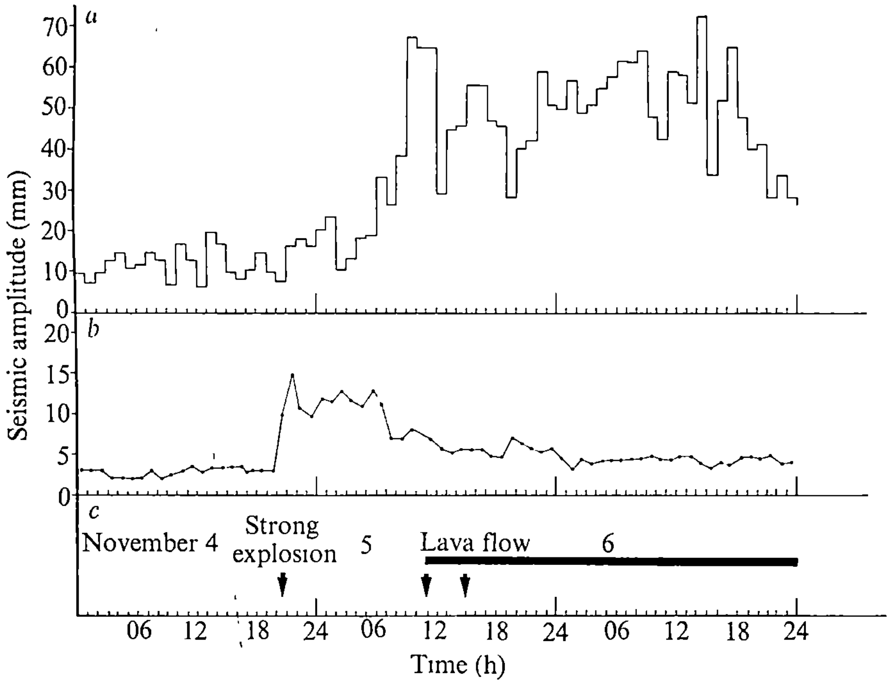


Fig. 3 *a*, Hourly frequencies of seismic events; *b*, relative amplitude of tremors; *c*, eruptive activity.

The earliest lavas produced were observed on November 5, 1975 at 1500. At that time the two flow fronts were at 400 m OD and 500 m OD on the Sciara del Fuoco, respectively. The first flow originated from a new bocca at 700 m OD on the Sciara del Fuoco; the second flowed from a collapse of the crater rim originating from a vent within the main crater close to the lip (Fig. 1). This flow stopped on November 6. The flow from the bocca on the Sciara del Fuoco reached the sea on November 5 at 1800.

The lava flow down the Sciara was 10 m wide, but at the Sciara base where the lava flowed into the sea, the flow formed a delta-like body with a front about 100 m wide. Only a small part of this material was molten lava, flanked with fragments of lava pushed aside during flow along the Sciara. The effusive activity stopped on November 24.

The explosive activity reached its peak between November 5 and November 7 (when more than one explosion occurred every second). Two of the vents inside the elongated crater (Crater 1, Fig. 2*d*) were very active. Also active were vents 4 and 5. Crater 1 was characterised by fire fountaining and explosions. The explosions threw lava fragments and scoriae to a maximum height of 700 m and there was rumbling within the crater. Vent 5 was also characterised by explosions with maximum projectile heights of about 400 m. The explosions from Vent 4 ejected ash to about 800 m above the crater lip. During this period of activity 65% of the explosions were from Crater 1 and 21% and 12%, respectively, from Vents 4 and 5. On November 10 and 11 the ejection of ash had replaced the ejection of molten fragmenta and scoriae. Only two craters were active, Crater 1, in which 52% of the explosions occurred, and Crater 5, in which 22% of the explosions occurred; the other 26% was represented by weak explosions (or very shallow earthquakes?) recorded only on the seismograph at Ginostra. On November 13 only the ejection of ash occurred from Craters 1 and 5.

The seismic activity of Stromboli Volcano is recorded at a station at Ginostra, located about 2 km west of the crater plain. The station includes a 1-Hz vertical geophone, and the amplified signal is sent by telemetry to the Osservatorio Geofisico di Lipari. There, the signal is recorded on magnetic tape and monitored. Generally, the seismic activity of Stromboli comprises single seismic events superimposed on a continuous microtremor. On the evening of November 4, 1975, the amplitude of volcanic tremor increased sharply from 2000, and suddenly decreased at 0700 on November 5 (Fig. 3*b*). At that time the hourly frequencies of the seismic events increased (Fig. 3*a*). Figure 3 shows that the increase of the volcanic tremor began 11 h before the beginning of eruptive paroxysm. On the morning of November 7, in order to determine the nature of the single events recorded, observations were conducted for 1 h at the crater plain. Around 99% of the events recorded were related to observed explosions. During later observations (November 10, 11, and 13) the proportions of seismic events related to explosions were 65%, 42% and 35%, respectively. This change must be related to the fact that the level of the magmatic column became progressively lower. In fact, after November 10 the products of explosion appeared on the crater lip more than 10 s after the seismic event was recorded.

The seismic record during this recent activity of Stromboli confirms that the source of the seismic energy is located within the volcanic superstructure, that an inverse relationship between the amplitude of microtremors and number of events per unit time exists (Fig. 3), and that the seismic events can be clearly related to events in the crater. Such a clear relationship between events in the crater, and seismic activity recorded, suggests that major changes of crater activity can be detected by the existing seismic network.

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Sulphate–light scattering ratio as an index of the role of sulphur in tropospheric optics

THE optical properties of the non-cloudy atmosphere are determined by a fixed component, the molecular gases, and a variable component, the aerosol particles. The optical effects of these two components have been subject to speculation and scientific investigation for many years. The major features of molecular and particle optics are reasonably well understood[1–3] and instrumental techniques have been developed to measure extinction of light[4], scattering phase function[5,6] and the scattering component of extinction[7]. Few data or analyses have, however, been presented which consider the chemical nature of the aerosol in terms of its effects on radiative properties, although a few exceptions can be cited[8–12]. Experimental evidence quantitatively linking the chemical composition of aerosol particles with the macroscopic optical consequences is sparse, but here we mention one approach relating chemical and optical measurements on regional scale air masses and present preliminary data.

Measurements[13,14] have shown that the aerosol particle volume distribution is usually bimodal, and that the two modes are approximately log normal, are of mass mean diameters 0.4–0.8 μm and 8–20 μm and geometric standard deviations of about 2. Mie calculations show that the small particle mode usually dominates the optical scattering. Further, the measured particle volume concentration in the range 0.1–1.0 μm is often highly correlated to the extinction due to scattering by particles as measured with an integrating nephelometer with a correlation coefficient as high as 0.95 (ref. 14). Chemical analyses have shown that the small particle mode is often dominated by few substances notably non-maritime sulphates, nitrates, ammonium and condensed organic material[8,15,16]. Measurements in and near St Louis, Missouri[17] showed that sulphates often accounted for $\geq 50\%$ of the mass of material in the diameter range $0.1 \geq D_p \geq 1$ μm.

Dry particle light scattering and the possibly dominant chemical quantity (sulphate compounds) seem the most useful parameters to measure. These measurements may be compared with other atmospheric data, with laboratory values for simulated systems and with Mie theory to provide insight into the fraction of optical effect attributable to sulphate components. The key ratio is that of sulphate concentration to light scattering extinction coefficient due to dry particles, $[SO_4^{2-}]/b_{sp}$, g m^{-2}.

Table 1 provides a summary of various schemes which can be used for this evaluation of the mass concentration/b_{sp} and/or $[SO_4^{2-}]/b_{sp}$ ratio. It should be noted, of course, that "sulphate" is not a compound itself and must have a cation. The lightest cation is H^+ and it is found in atmospheric particles some of the time. One of the heavier ions is NH_4^+ as in $(NH_4)_2SO_4$, which in also frequently found as an optically dominant component (that is, 50 mol %)[17]. Since the ratio of molecular weight of $(NH_4)_2SO_4$ to that of SO_4^{2-} is only about 1.5, and that of H_2SO_4 is almost 1, the use of SO_4^{2-} mass concentration alone for an index is warranted at the outset. This simplification, which is due, in many cases, to a lack of knowledge, of the cation associated with sulphate, must be kept in mind as it produces an inherent uncertainty of about ±25% in the ratio.

The data pairs for the ratio were obtained from aircraft flights, mainly over southern Sweden. An integrating nephelometer[23] was used to measure the extinction coefficient due to dry particles at a given wavelength (in most cases 450 nm) and a filter was used to obtain samples for postanalysis of the SO_4^{2-} content by the thorin method. Incoming air was heated to ~20 °C, providing relative humidities below 50%. Some flight paths and altitudes were selected beforehand to provide air from populated areas of continental Europe as part of the study by the Organization for Economic Cooperation and Development of sulphur transport across national boundaries *vis-à-vis* the problem of acidic rain (Long Range Transport of Air Pollutants project), whereas others were deliberately made at random. Most flights were at altitudes above 300–1,000 m and well inland (250 km) so that large particle ($D_p > 1$ μm) maritime sulphate concentrations should be small or nonexistent. The average sulphate concentrations ranged up to 35 μg m^{-3}, significantly higher than the maritime SO_4^{2-} in this area (typically less than 3–10 μg m^{-3} based on Cl^- analysis) and provided an adequate dynamic range for correlation calculations.

Table 2 includes all ratios of the $[SO_4^{2-}]$ and b_{sp}, and summarises the correlation calculations.

(1) The $[SO_4^{2-}]/b_{sp}$ ratio from the overall regression is 0.2 g m^{-2} which is comparable with the measured and predicted ratios in Table 1 for the ratio of submicrometre mass concentration to b_{sp}.

(2) The correlation between $[SO_4^{2-}]$ and b_{sp} for all data is 0.73, indicating that a large fraction of the variance of b_{sp} can be associated with $[SO_4^{2-}]$.

(3) The correlation between $[SO_4^{2-}]$ and b_{sp} for individual days (that is, flights) ranges from $r=0.99$ to $r=0.24$. Five of nine cases with three or more data points had $r > 0.86$, whereas the rest had $r < 0.60$. Trajectory calculations showed that the

Table 1 Ratio of volume or mass concentration of submicrometre particles to light scattering

Basis	Modal volume ratio, v/b_{sp} (cm^3 m^{-2})	Modal mass ratio, m/b_{sp} (g m^{-2})	Volume or mass correlation to b_{sp}	Reference
(1) Measured volume concentration in diameter range $0.1 \geq D_p \geq 1.0$ μm and measured light scattering coefficient (b_{sp}), from several suburban locations in southern California	0.1	0.2*	0.95	14
2) Mie calculations using a log normal size distribution of geometric mean diameter by volume = 0.6 μm, geometric s.d = 2, refractive index = 1.5	0.15	0.3*	—	18
(3) Measured volume concentration with $0.1 \geq D_p \geq 1.0$ μm and measured b_{sp}, from Pasadena, California	0.08	0.16*	0.82	19
(4) Measured volume concentration with $D_p < 2$ μm in urban-influenced (urban district) and aged polluted cases	0.05–0.15	0.10–0.3*	—	14
(5) From direct measurement of total mass concentration and b_{sp}, estimation that half of mass is in optically active range $0.1 \leq D_p \leq 1.0$ μm	—	0.2	0.82	20
(6) From direct measurement of total submicrometre mass concentration and b_{sp}, from St Louis, Missouri	—	0.22	—	15, 17
(7) $[SO_4^{2-}]/b_{sp}$, from St Louis, Missouri	—	0.1	—	15, 17
(8) Calculated extinction coefficient for a model aerosol of $(NH_4)_2SO_4$ at 70% r.h.	—	0.1 up to 0.2	—	21
(9) Laboratory aerosol of $(NH_4)_2SO_4$ generated by sparger $r_{gm} = 0.6$ μm, $\sigma_g = 1.43$	—	0.097+0.007	—	22

*Particle density assumed to be 2 g cm^{-3}

Table 2 Measured ratios and correlations

Year	Month	Day	Local time	N	r	$[SO_4^{2-}]/b_{sp}$§ (g m^{-2})	Trajectory
1973	May	9	1300–1515	7 (6)*	0.54 (0.96)*	0.21	‖
	May	23	1245–1500	7	0.99	0.11	‖
	June	13	1400–1600	5	0.97	0.15	‖
	June	28	1345–1430	2			
	July	4	1420–1650	4 (3)*	0.60 (0.93)*	0.16*	‖
	December	20	1500–2100	9	0.93	0.38	‖
1974	January	3	1214–1650	3	0.99	0.49	
	March	14	1210–1410	8	0.50	0.07	‖
	March	19	1025–1100	2		0.10	
	March	21	1340–1430	2		0.45	
	May	3	1045–1245	6	0.48	0.15	¶
	May	16	1115–1330	8	0.35	0.17	¶
	June	5	1030–1230	10	0.24†	0.06	¶
	May	28	1227–1437	4	0.86‡	0.08	
	Total			77	0.73	0.2	

N, Number of samples.
r, Linear correlation coefficient.
*If one data point is omitted.
†SO_4^{2-} concentration is too low to permit accurate readings.
‡Measurements at 530 nm.
§Least square estimate (calculated as $k = \Sigma ([SO_4^{2-}]b_{sp})/\Sigma(b_{sp})^2$.
‖Trajectory from south-east, south, south-west, west.
¶Trajectory from north-west, north, north-east, east.

high correlation cases occurred with flow from the south-east, south and south-west whereas the low correlation cases were from the north-west, north and north-east.

(4) The $[SO_4^{2-}]/b_{sp}$ ratio is comparable with that found in St Louis, Missouri.

(5) If the size distribution were known, more definite conclusions could be made regarding the fraction of b_{sp} due to $[SO_4^{2-}]$. Such measurements need to be made in the future, especially resolving $[SO_4^{2-}]$ as a function of size.

(6) The cases in which the correlation coefficient was low, and the cases where $[SO_4^{2-}]/b_{sp} \leqslant 0.2$ g m^{-2} do not fit the simple model of $[SO_4^{2-}]$ dominance of b_{sp}. In the case of a ratio greater than 0.2 g m^{-2}, it seems possible that large particle SO_4^{2-} was present. Further sampling and chemical analyses are needed to explain these cases. There were very few cases of $[SO_4^{2-}]/b_{sp} < 0.1$ g m^{-2}, so that there was nearly always sufficient SO_4^{2-} to account for the observed b_{sp}. When the SO_4^{2-}/b_{sp} was 0.2 g m^{-2}, assuming a particle size distribution similar to that found by Whitby[16] (log normal, geometric standard deviation = 2, mass mean radius = 0.3 μm) leads to the conclusion that much, and perhaps most, of the light scattering was due to SO_4^{2-}. If the measured ratio was ~0.1 g m^{-2}, then perhaps half of the optical effect can be attributed to SO_4^{2-} (assuming constant size distribution, and so on). Further, the two separate analyses (regression analysis and comparison of measured ratios with those from theory and other experiments) are consistent with this same conclusion.

Since the SO_4^{2-} in southern Scandinavia in the selected meteorological conditions probably arises from the oxidation of man-made SO_2 (ref. 24), these measurements tend to indicate that SO_4^{2-} is not only involved in rain chemistry but also in the aerosol and its optical consequences.

Using this approach, it is interesting to consider the $[SO_4^{2-}]/b_{sp}$ ratio as an index of the role of sulphur in tropospheric optics and (since man is rapidly changing the sulphur cycle) also as an index of the effects of human activity on atmospheric optics. Before we can be certain, however, more data will be needed, especially with regard to the non-human, natural sources of submicrometre SO_4^{2-} aerosol. Until such time as these data exist, we feel that the study of the $[SO_4^{2-}]/b_{sp}$ ratio and its variations is warranted to explore and test these hypotheses.

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Prevailing winds in lower thermosphere

WINDS in the upper atmosphere have been measured at Adelaide, South Australia (35°S) by radio observations of drifting meteor trails, using a combined continuous wave and pulse technique[1]. The systematic features of the winds can be described in terms of a prevailing component and 24-, 12- and 8-h periodic components. Here we are concerned primarily with the behaviour of the prevailing component in the height range 75–105 km for the six years from June 1966 to June 1972.

The observations were usually carried out on four successive days near the middle of each month. Each quarter the observing interval was extended to 7 d, and on a few occasions to 20 d. An average of 250 usable echoes giving drift measurements were recorded each day. The analysis was carried out using a least squares fitting method developed by Groves[2]. For each day this gives the most probable height profiles of the prevailing and periodic wind components. The precision in height is ±2 km.

Figure 1 shows isotachs of the zonal and meridional winds. The zonal wind has a very regular annual pattern and exhibits two distinct circulation regimes. Below 85 km eastward winds occur in winter and westward winds in early summer, corresponding to the monsoonal type circulation that dominates the air motion in the mesosphere and upper stratosphere. Above 90 km the main features of the circulation are a strong eastward flow in summer, a westward flow in winter and early spring, and relatively large gradients at the solstices. A rapid build-up of eastward flow occurs at upper levels during October and November reaching speeds as high as 80 m s^{-1} at 100 km in December. In some years a second but weaker maximum in the upper circulation pattern occurs about March. The superposition of the two zonal circulation systems produces a complex behaviour near 90 km where the flow is predominantly eastward, and lacks any marked annual variation as illustrated in Table 1.

The reversals of zonal flow that occur at the lower levels are of short duration, typically ~ 1 month. The times of reversals show a phase retardation with height of 0.3–1 d km^{-1}. On three occasions, during the months of November, 1966, 1969 and 1970 the reversals extended up to 100 km and on two occasions merged with the westward flow of the upper pattern just before its changeover to the strong summer circulation.

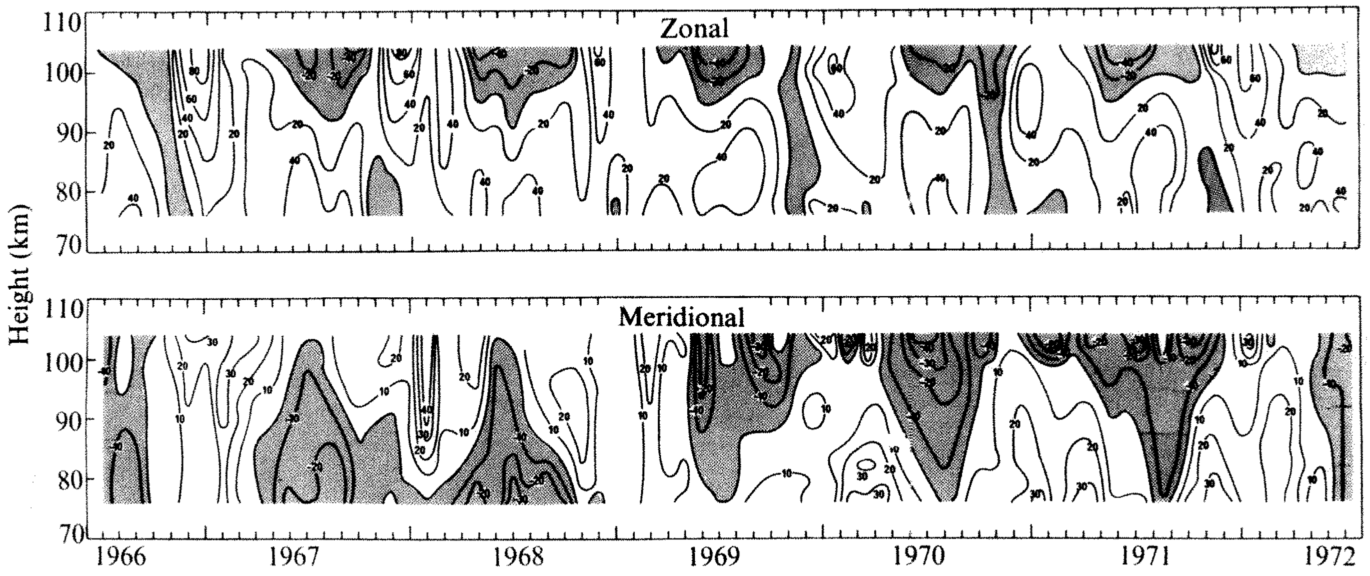
Table 1 Amplitudes and phases of the annual and semi-annual waves

Height	Annual		Semi-annual	
	Zonal wind			
(km)	Amplitude (m s^{-1})	Phase (date)	Amplitude (m s^{-1})	Phase (date)
82	16	May 25	7	January 10
90	6	February 21	6	December 26
98	25	December 28	9	December 15
	Meridional wind			
(km)	Amplitude (m s^{-1})	Phase (date)	Amplitude (m s^{-1})	Phase (date)
82	14	January 4	4	March 15
90	13	December 28	4	March 6
98	12	December 28	3	February 24

The meridional prevailing winds exhibit annual reversals that are symmetrical in time with respect to the solstices, although the time of a reversal can change with height as rapidly as 7 d km^{-1}. The maximum southward flow occurs about the time of the winter solstice at all heights while the northward flow usually exhibits two maxima close to midsummer. Extreme meridional speeds rarely exceed 30 m s^{-1}. The pattern of the meridional flow changed in early 1969 so that the predominantly northward flow that had occurred at the upper levels since mid-1966 became a southward flow of almost equal strength. This change is reflected in the gradient of the meridional wind as shown in Fig. 2. The gradient values have been calculated at the 90-km level but are generally typical of the whole height range under investigation. The changeover commenced about October 1968 and was completed by April 1969. Early in 1973 a return to positive meridional gradients occurred. There is no evidence of any substantial complementary changes in the zonal flow at these times.

The variability in the meridional gradient during the years 1969–72 is of the order of the mean value of the gradient so that the extremes range between 0 and −2 m s^{-1} km^{-1}. The times of occurrence of the maxima and minima do not show any seasonal dependence. In general the gradients that occur in the meridional flow are much smaller than those in the zonal flow. At 90 km the zonal gradients range from a mean value of 2.2 m s^{-1} km^{-1} in summer to −3.7 s^{-1} km^{-1} in winter. An extreme value of −5.5 m s^{-1} km^{-1} occurred during the winter of 1969. Even higher values occur near 100 km.

Fig. 1 Height–time cross sections of the zonal and meridional prevailing winds for the period June 1966–June 1972. The shaded areas represent winds toward the west and toward the south. Speeds in m s^{-1}.



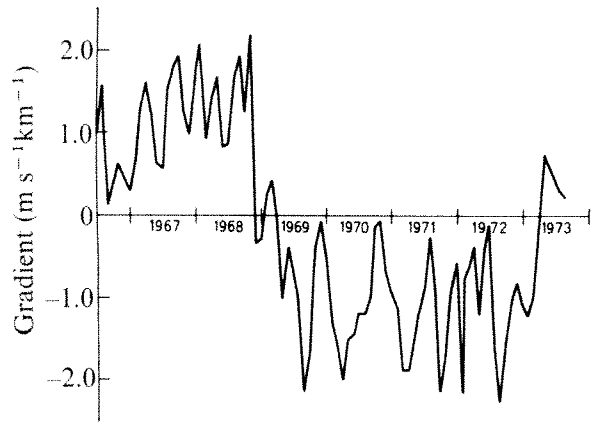


Fig. 2 Vertical gradient of the meridional prevailing wind measured at 90 km showing a sudden change in early 1969.

The annual and semi-annual variation in the prevailing winds have been determined for the period June 1966–June 1972, and the amplitudes and phases (times of maximum eastward or northward flow) at the heights 82, 90 and 98 km, are given in Table 1.

The phase of the annual oscillation in the zonal winds at 82 km is consistent with the phase of this oscillation in the lower mesosphere determined from rocketsonde data[3]. These results show that at 35°S and at a height of 65 km, maximum eastward flow occurs in late June and tends to be earlier with increasing height. The amplitude of the annual wave deduced from the rocketsonde data is 60–70 m s^{-1} at 65 km, whereas the meteor drift observations indicate an amplitude of 16 m s^{-1} at 82 km. In spite of this rapid fall off in amplitude above 70 km, the consistency of the phase between the heights of 60 and 80 km supports the earlier comment that the mesospheric circumpolar vortex extends to a height of ~ 85 km.

The zonal semi-annual oscillation at 82 km has its maximum eastward flow on January 10; this is about three months earlier than the time of occurrence of maximum eastward flow at 65 km, determined from the rocketsonde data. Such a rapid phase shift with height is not consistent with the relatively slow change in phase below 65 km and requires further investigation.

At a height of 98 km the annual oscillation in the zonal wind has an amplitude of 25 m s^{-1} and its strength increases with height. The time of maximum eastward flow occurs close to mid-summer. This value of phase is maintained up to the limit of the data at 104 km and is probably typical of this oscillation in the lower thermosphere.

In addition to the annual and semi-annual waves in the zonal winds, oscillations with periods of four months and three months occur with significant amplitudes. These reflect the rapid increase of the zonal wind strength during the months October to December. The meridional prevailing winds are dominated by the annual oscillation whose maximum northward flow occurs almost simultaneously at all levels, a few days after the summer solstice. It is of interest to note that this annual oscillation was essentially unaffected by the major change in the mean meridional flow that occurred in 1969.

Table 2 Standard deviations of the zonal and meridional prevailing winds (speeds in m s^{-1})

Height (km)	Zonal Summer	Zonal Autumn	Zonal Winter	Zonal Spring
82	16	15	14	14
90	12	13	15	18
98	13	17	17	18

Height (km)	Meridional Summer 1966–69	Summer 1969–72	Autumn 1966–69	Autumn 1969–72	Winter 1966–72	Spring 1966–72
82	27	10	16	11	11	11
90	28	12	17	12	10	10
98	25	14	18	15	16	12

The significance of the mean meridional flow, its long term variations and annual oscillation is open to conjecture. One possibility is that the flow arises from a circumpolar standing wave with a phase variation with height. An annual variation in the phase constant of the wave would account for the annual variation in the merdional winds at any level. Further, a long term change in the phase constant of the whole wave system could reverse the gradient of the winds as is observed. It has been shown[4] that such a standing wave can transport significant quantities of heat towards or away from the polar regions.

An alternative possibility is that the observed meridional flow is typical of the mean meridional flow across a circle of latitude. This situation has been considered by Ebel[5] who has shown that such flows imply meridional cells with vertical velocities of the order of a few cm s^{-1}, and momentum sources and sinks of ~ 100 ms^{-1} d^{-1} per unit mass. The strength and direction of flow in the cells would undergo annual changes, while long term changes in the observed meridional winds could be produced by changes in the latitude of the cell boundaries. If the latitudinal distribution of the zonal and meridional winds are known, it is also possible to calculate the strengths of the heat and momentum sources required to drive this meridional circulation[6]. The actual situation is likely to be a combination of both mean meridional flow and circumpolar standing waves. The contribution which each makes to the observed north–south winds must await simultaneous observations at several sites around a circle of latitude.

On a limited number of occasions when observations extended continuously for ten days or more, slow and regular changes in the strength of the zonal and meridional winds have been observed. The magnitude of the variation is comparable to the speed of the mean flow and the quasi-period is ~ 10–15 d. This transient motion occurs at all times of the year and is interpreted as planetary waves. The intensity of the variability produced by this long wave activity has been determined by calculating, for each season, the s.d. of the observed daily values of zonal and meridional flow from the mean monthly values. The results are given in Table 2. The strength of the zonal variability is independent of the season at 82 km, although above 90 km it is marginally smaller in summer. The pattern of meridional variability showed a marked change in 1969. For the period 1969–72, its magnitude is essentially independent of season and shows only a marginal increase with height: for the period 1966–69 the variability is two to three times larger in summer than in winter. Although the mechanism for this change in the strength of the variability is not known, it is clearly associated with the reversal of the gradient of the mean meridional flow which occurred in early 1969.

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Mercury in Mersey estuary sediments, and the analytical procedure for total mercury

THE Mersey estuary consists of a wide, shallow upper region, separated from the sea by deeper 'narrows'. The upper estuary (Fig. 1) is an area of continuous accretion of sediment, observed since the mid-nineteenth century with occasional short periods of depletion. The main freshwater inflows are the River Mersey itself, flowing over Howley Weir, and the Manchester Ship Canal, entering the estuary at Eastham Locks. The River Weaver flows into the Manchester Ship Canal at Weston[1]. The area is industrialised and heavily populated and the estuary receives both domestic sewage from surrounding towns and industrial effluent[1,2]. We are aware of no published data for mercury concentrations in the sediments of the estuary, and have undertaken such measurements. Samples were obtained from the places shown in Fig. 1. We analysed 136 samples for total mercury and the results presented in Fig. 1 and in Table 1 are compared with other data. The levels found are, in general, somewhat higher than in other, more limited, surveys.

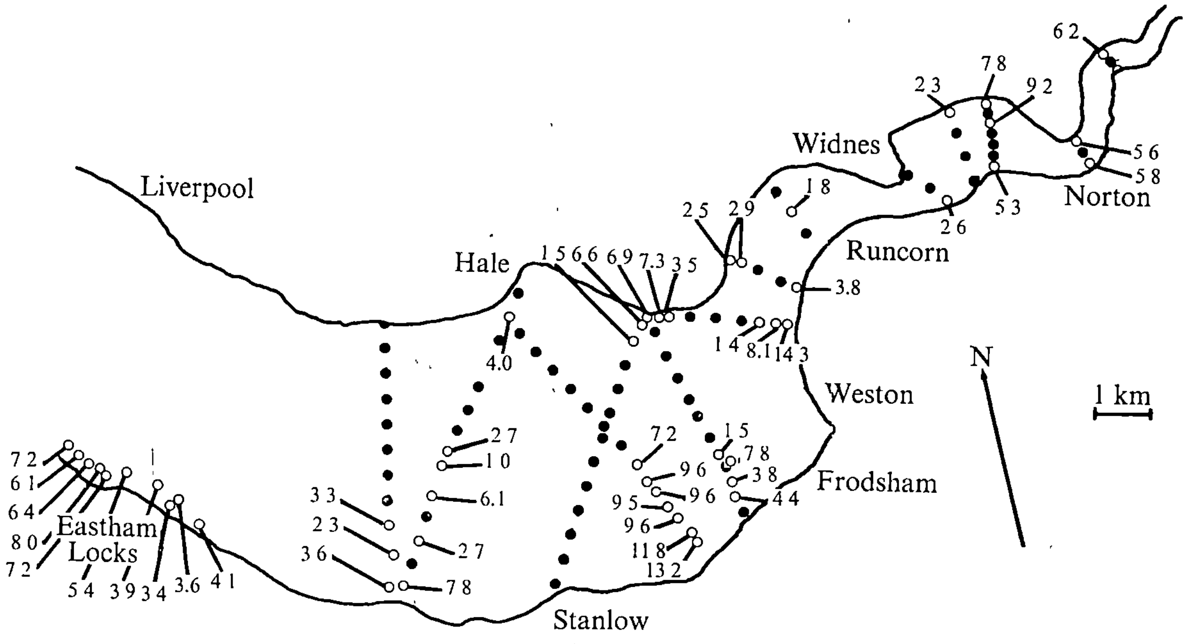
The samples were obtained as follows: the top 5 cm of the sediment were sampled, and 10–20 g were taken at each point. The samples were stored frozen, in glass bottles, until analysis. Total mercury was determined by cold vapour atomic absorption spectrophotometry following digestion in aqua regia, and oxidation with a mixture of potassium dichromate and potassium persulphate[3,4]. This was found to be superior to the potassium permanganate mixture suggested previously[3,5], as this has a tendency to deposit oxides of manganese on the vessel walls, trapping a proportion of the mercury. Replicate analyses of 10 identical test samples gave a mean value of 2.46 μg g^{-1}, and a r.s.d. of 3.3%. The detection limit (the mean of the blank, plus twice the s.d. of the blank) was ~0.01 μg g^{-1} in the sediment. Portions of the samples were dried to determine the water content, and all results are quoted as μg per g of dry sediment.

Table 1 Mercury concentrations in surface sediments

Sample Area	Number of Samples	Total mercury (µg g^{-1}) Mean	Min	Max	Reference
River Mersey, England	136	2.23	0.01	14.30	this work
Swansea Bay, Wales	55			1.60	7
Swansea Bay, Wales	11	0.19	0.04	0.76	8
La Have River, Nova Scotia	5	0.34	0.09	1.06	9
Lake Windermere, England	1	1.05			10
Lake Superior	405	0.08		0.58	11
Lake Michigan (South)	31	0.15	0.03	0.38	11
Lake Michigan (North Channel)	55	0.15	0.08	1.11	11
Lake Huron (Georgian Bay)	117	0.26	0.01	9.50	11
Lake St Clair	55	0.63	0.07	2.57	11
Lake Erie	243	0.61	0.13	7.49	11
Lake Ontario	248	0.66	0.03	2 10	11
Pallanza Bay (Lake Maggiore), Italy	34	7.65	0.24	20.10	11
Everglades, Florida	10	0.62	0.12	1.50	12, 13
Mobile Bay	4	0.38	0.22	0.60	12, 13
Mississippi River	4	0.38	0.08	0.57	13
Lake Huleh, Israel	15	1.07			14
Southampton Water, England	3	0.30	0.19	0.64	15*
Thames Estuary, England	15	0.132	0.02	0.49	16

*Mean value including two 10-cm core samples = 1.60 p.p.m.

Fig. 1 Total mercury levels in the upper Mersey estuary. ● indicates < 1µg g^{-1}.



The mean value in the 136 samples was 2.23 μg g^{-1}. The maximum value recorded was 14.3 μg g^{-1}, and 23 samples were below the detection limit. High values occurred in those sediments consisting of fine silt, with a large amount of organic material present. This type of sediment is found along the edges of the water course. Lowest values were found in the relatively clean sandy material from the fast running tidal channels of the centre of the estuary. Figure 1 shows Hg concentrations from sampling points with levels >1 μg g^{-1}. Sampling points with levels <1 μg g^{-1} are shown as dots. These results indicate three main areas with significantly elevated total mercury concentrations; at Norton, Weston, and Stanlow. Distribution of mercury in the sediments reflects the position and movement of the fine silt in the estuary, and a high mercury level is not necessarily indicative of a mercury input at that point.

Several studies of total mercury concentrations in sediments, but mainly relating to North America, have been carried out and comparison with our work is made in Table 1.

It was not possible to analyse all samples immediately and a report that if a sample of a river sediment containing mercury is taken and stored at −15 °C, 50% of the total mercury present originally is lost after 7 d (ref. 6), was therefore relevant to our work. We investigated this alleged loss of mercury from frozen sediments to determine the effect of storage in various conditions on the levels of total mercury found when analysis was carried out.

We found that total mercury does not seem to be lost in significant quantities from samples stored at −20 °C, −6 °C and even at room temperature (allowing for natural temperature variations). This does not agree with previous work, but a possible explanation is that in Daughdrill's samples[6] the mercury was present in the form of methyl mercury or that mercuric sulphide was being formed. The form of the sampling was as follows: a bulk sample was taken initially and immediately homogenised and divided into a number of smaller samples. These were frozen with solid carbon dioxide and taken to the laboratory where the first analysis was made the next day (day 0, corresponding to the true environmental level). The data presented in Fig. 2 are of importance for analytical work since they indicate that sediments containing mercury may be stored even at room temperature for at least 9 weeks and still give reliable values for total mercury content.

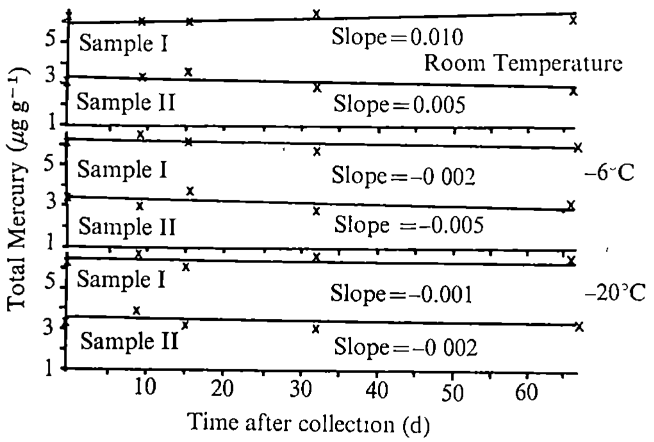


Fig. 2 Repeated analysis of sediment for total mercury

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Aliphatic amines in the Murchison meteorite

ANALYSES of the organic compounds found in the Murchison meteorite, a C2 chondrite that fell in Australia in 1969, have provided much new information about extraterrestrial organic chemical evolution. The soluble fraction of this meteorite has been shown to contain aromatic and aliphatic hydrocarbons[1–4], mono-[5] and dicarboxylic acids[6] and various nitrogen-containing organic compounds. The latter include both amino acids[2,3,7–9] and nitrogen-heterocyclic compounds[10–11]. The presence of amines, ureas and amides has been suggested[11], although no individual compounds from these classes have been identified. Here we report the determination of aliphatic amines in water extracts of the Murchison meteorite.

The Murchison samples used in this work were clean interior specimens obtained from the Center for Meteorite Studies, Arizona State University. All glassware was acid-washed and heated in an oven at the annealing temperature of glass before use. Water for extraction was distilled in an all-glass distilling apparatus from alkaline potassium permanganate. The Murchison fragments were crushed in a clean mortar and transferred to the extraction vessel. One set of samples was extracted in a round bottomed flask under reflux conditions as described previously[7]. These samples were subsequently hydrolysed in 6 N HCl. Another set of Murchison samples was extracted in sealed ampoules at 120 °C for 4 d.

The amines of the aqueous extracts were analysed by gas chromatography both as the free amines (Fig. 1*a*) and as 2,4-dinitrophenyl (DNP) derivatives (Fig. 1*b*). Methylamine, ethylamine, diethylamine, isopropylamine, *n*-butylamine, *sec*-butylamine, isobutylamine, and *tert*-butylamine were identified as the free amines. Diethylamine and *n*-propylamine could not be identified unambiguously by this method because of incomplete resolution. Peaks appear on the chromatogram at retention times that do not correspond to those of any of the known amines. This is not surprising since the amines were not separated from other organic material in the meteorite extract before chromatography. But most of the interfering low molecular weight compounds would be expected to have been volatilised during reduction in volume of the solution, and other compounds known to be present in the extracts such as fatty acids and dicarboxylic acids should have been trapped as salts in the injection port. Injection of an 18-component amino acid standard solution (Bio Rad Laboratories) did not cause elution of any peaks corresponding to known amines although a few peaks were apparent that matched unidentified peaks in the Murchison extract.

In order to strengthen the evidence for amines, gas chromatographic separation of the DNP derivatives was carried out[14]. In Fig. 1*b* peaks are seen corresponding to the DNP derivatives of ethylamine, dimethylamine and diethylamine, as well as at the positions of the unresolved pairs, isopropylamine–*tert*-butylamine, methylamine–*sec*-butylamine, and *n*-propylamine–isobutylamine. A large dinitroaniline (DNA) peak is observed in addition to several unknowns.

Primary amines can be readily determined with an amino acid analyser by extending the final elution step (J.R.C. and P. E. Hare, unpublished). This method was used for analysis of the Murchison amines and the chromatogram is shown in Fig. 2. Well resolved peaks are seen corresponding to methylamine, ethylamine, isopropylamine, *tert*-butylamine, *n*-propylamine and *n*-butylamine. An unresolved peak is seen corresponding to *sec*-butylamine and/or isobutylamine. Secondary amines are not detected with this system as a result of their lack of reactivity with the detection reagent. An aqueous extract of the meteorite that had not been subjected to acid hydrolysis was also chromatographed. The amounts of each amine found, both before and after acid hydrolysis, are given in Table 1.

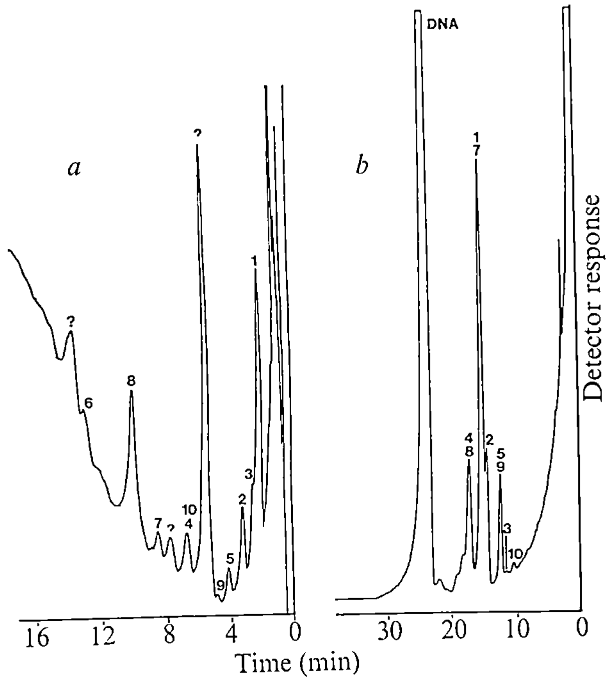

Fig. 1 Gas chromatographic analysis of meteorite amines. The peaks corresponding to known amines or DNP amines are identified by number as in Table 1. *a*, Gas chromatography of the free amines. An extract derived from approximately 0.5 g of the Murchison meteorite was acidified and evaporated to near dryness with gentle heating under a stream of purified nitrogen. The resulting solution was injected directly into the injection port of a Perkin-Elmer 3920 gas chromatograph. The injection port was packed with 25% potassium hydroxide on 80/100 mesh Chromosorb W in order to liberate the free amines from the hydrochloride salts[12]. The gas chromatograph contained a 12 foot×1/8 inch stainless steel column packed with 20% UCON LB 550-X plus 10% KOH on 120/140 mesh Chromosorb W and was temperature programmed from 60 °C to 110 °C at 4 °C min^{-1} with a helium flow rate of 50 ml min^{-1}. *b*, Gas chromatography of the DNP amines. The amine DNP derivatives were prepared from 2 ml of meteorite extract (0.8 g meteorite) by reaction with sodium dinitrobenzenesulphonate[13]. The reaction time was lengthened to enhance the formation of DNP derivatives of the secondary amines. The DNP amines were extracted into benzene and washed with a saturated borax solution. The volume of the benzene layer was reduced to a few μl and analysed on a 6 foot×1/8 inch stainless steel column packed with 7% XE-60 on 110/120 Anakrom ABS.

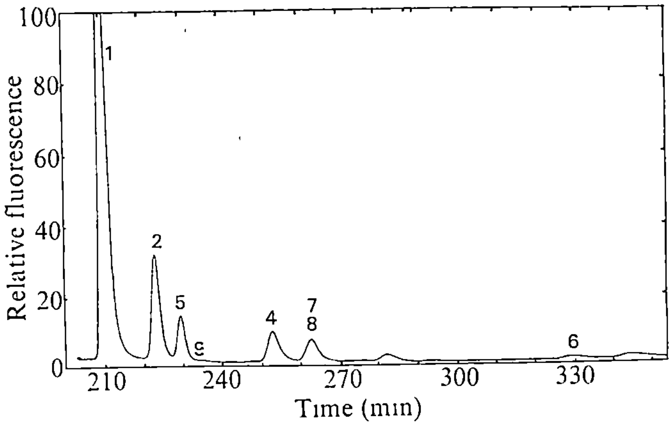

Fig. 2 Ion exchange chromatographic analysis of meteorite amines. The peaks corresponding to known amines are identified by number as in Table 1. An acid-hydrolysed water extract from 0.09 g Murchison meteorite was applied to a 0.20×25 cm DC4A resin (Durrum Chemical Co., Palo Alto) column. Separation of amino acids in the sample was achieved by sequential elution of the column with citrate buffers (0.2 M sodium ion) of increasing *p*H. Basic amino acids, followed by amines, were brought off the column by continuing the elution with a 0.032 M borate buffer, *p*H 10.7, which had been brought to 0.23 M in sodium ion by the addition of NaCl. Amino acids and amines were detected fluorometrically after reaction with *o*-phthalaldehyde[15]. Only the latter part of the overall chromatogram is reproduced above.

The results summarised in Table 1 give evidence for the presence in water extracts of the Murchison meteorite of all of the possible primary aliphatic monoamines (eight) with fewer than five carbon atoms. Five of these amines could be identified unambiguously in at least two of the three chromatographic systems used. Two of the seven possible secondary or tertiary aliphatic monoamines were identified. Chromatographic standards were lacking for the remaining five amines of this type and they went unrecognised, if present.

The primary amines that could be identified in the aqueous extract total 80 nmol per g meteorite. It is interesting to note that these compounds, some of which are quite volatile, have not been lost during the travel of the meteorite in space. They thus seem to be chemically or physically trapped in the meteorite.

Two similarities are apparent between the water-extractable amines and amino acids of the Murchison meteorite: (1) The level at which amines are present declines sharply with increasing carbon number in the homologous series, methylamine, ethylamine, *n*-propylamine, *n*-butylamine. Although the decline in content is not as sharp, the same is true within the amino acid series,

Table 1 Chromatographic identification of amines

Amine	Gas chromatography		Ion exchange chromatography (nmol per g meteorite)	
	Free amine	DNP amine	Extract	Hydrolysed extract
1 Methylamine	+	+*	49	143
2 Ethylamine	+	+	14	30
3 Dimethylamine	+	+	†	†
4 *n*-Propylamine	+*	+*	5	10
5 Isopropylamine	+	+*	9	13
6 *n*-Butylamine	+		0	3
7 *sec*-Butylamine	+	+*	+*	+*
8 Isobutylamine	+	+*	+*	+*
9 *tert*-Butylamine	+	+*	3	3
10 Diethylamine	+*	+	†	†

*Unambiguous identification not possible because of incomplete resolution.

†Secondary amines not detectable with *o*-phthalaldehyde

glycine, alanine, α-amino-*n*-butyric acid, norvaline, norleucine. These quantitative relationships are consistent with a chemical synthesis in which a simple carbon compound, methane, for example, is the precursor of meteorite amines and amino acids. (2) The amine content of the aqueous meteorite extract increases by over 100% on acid hydrolysis. A similar observation has been made with respect to the amino acids in aqueous extracts of both the Murchison and Murray meteorites[7]. Thus both amines and amino acids are extracted from the meteorite as such, as well as in the form of acid-hydrolysable derivative or precursor species.

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Antiquity of man in America indicated by radiometric dates on the Yuha burial site

MUCH evidence suggests that man was present in the Western Hemisphere before 12,000 yr ago, but the case has remained less than conclusive[1]. In some situations, the geological age of the site is reasonably well established but the association or nature of the artefacts is questionable[2,3]. In other cases, museum specimens of human bones dated by radiocarbon analysis of collagen lack desirable information concerning site location, geology, and stratigraphy even though the accuracy of their absolute ages seems valid[4–6]. We report here the results of radiometric dates of the Yuha burial site from Imperial County, California, for which the geology and stratigraphy have been documented and reported in detail[7].

Claims of great antiquity on human bones based on amino acid racemisation[8] also suffer from shortcomings. The applicability of this technique by itself to bones where burial history is unknown is doubtful, and successful application of this technique requires a known temperature history of the bone, or, alternatively, a radiocarbon calibration point[9–13].

The Yuha site was discovered and excavated in 1971 and consisted of a partially preserved skeleton buried 35 cm below the surface. The burial was located on a dissected alluvial fan at the edge of a now dry pluvial lake, and was covered by a cairn of local cobbles and boulders. Flaked stone artefacts of a unifacial lower lithic industry were associated with the skeleton[7]. Subsequent to the time of burial, soil caliche precipitated, cementing the upper 15–60 cm of the entire alluvial deposit, coating the bones, the buried cairn boulders, and the underside of the exposed boulders.

A sample of the caliche coating the underside of one of the exposed cairn boulders (sample 103) and a sample of caliche coating one of the bone fragments (sample 104) were chosen for radiocarbon analysis. The results indicate ages of 22,125 ± 400 yr BP for sample 103 (R.P., UCLA2600) and 21,500 ± 1,000 yr BP for 104 (Geochron Laboratories, Cambridge, Massachusetts, GX2674). A modified ^{230}Th dating technique for application to soil caliches has been developed (T. L. Ku, W. B. Bull and K. G. Knauss, unpublished), but the technique can be successful only when corrections can be made for the detrital impurities. This was possible only for sample 103, which yielded an absolute age of 19,000 ± 3,000 yr BP, in good agreement with the radiocarbon results. In as much as the details of the ^{230}Th technique are yet to be published, however, the case for the antiquity of the Yuha skeleton must rest heavily on the radiocarbon results.

In proper conditions and with careful sample selection radiocarbon dates on soil caliches from arid zone palaeosols can indicate the true age of soil formation[14,15]. The Mungo burial in Australia represents essentially the same arid zone setting, environment, and time frame as the Yuha burial. Radiocarbon dates of soil caliche associated with this burial reflect the true age of local caliche pedogenesis[16].

A lone radiocarbon date on soil caliche, however, is always suspect because of possible contamination by younger organic matter in the soil or by older or 'dead' carbon from detrital limestone. Random or scattered apparent ages would result from such contamination, however, if more than one sample were analysed from a small area, since contamination is unlikely to be homogeneous over any distance. The close agreement among our samples with respect to age strongly suggests they are free from such contamination and represent the true age of the caliche precipitation. The ^{230}Th date supports this conclusion.

These results compare favourably with a date of $2{,}4000\pm^{2{,}000}_{1{,}000}$ yr BP (sample 401, ref. 7 and Geochron GX2722) obtained on soil caliche at the same elevation on the alluvial fan (134 m above sea level, but at a distance of 2 km from the burial). Similar caliches taken at successive distances up the slope from the burial and at higher elevations in the fan (250–300 m) yield successively older ages of 27,000–30,000 yr BP (ref. 7), indicating that caliche pedogenesis was progressive, beginning at the head of the fan and progressing down slope as the water table receded in response to desiccation of the pluvial lake. The original base level of the fan itself was apparently the high stand of the pluvial lake, at 45 m elevation, dated by lake shore tufa and freshwater snails at 37,400 yr BP (ref. 7).

In as much as the time of burial predates the precipitation of local caliche, our results yield a minimum age for the Yuha burial. Absolute dating of the skeleton itself by the collagen–radiocarbon technique will be carried out, but will require destruction of most of the skeletal material, since preliminary microanalytical analyses yielded a nitrogen content of only 0.68%. Consequently, such dating must await the completion of physical anthropological studies and construction of casts of each bone. With two A_2 dates the absolute chronological position of the Yuha hominid around 22,000 yr BP or older seems to be positively affirmed[17].

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Estimates of speeds of dinosaurs

THE faster an animal walks or runs the longer, in general, are its strides. Many dinosaur tracks have been found from which stride lengths can be measured[1]. I have now obtained a relationship between speed, stride length and body size from observations of living animals and applied this to dinosaurs to achieve estimates of their speeds. The estimated speeds are rather low—between 1.0 and 3.6 m s^{-1}.

Estimates of the speeds of large dinosaurs must depend on data from smaller animals. A means of allowing for differences in size is offered by the theory of physical similarity[2].

If meaningful comparisons are to be made between animals of different sizes, an appropriate non-dimensional parameter is needed to serve as a criterion for physical similarity. The most familiar parameter of this type is the Reynolds number which applies in situations where inertia and viscous forces interact and is very important in aerodynamics. Less familiar to biologists is the Froude number, which applies to any situation where inertia and gravity interact, and is important in nautical engineering. The Froude number is u^2/gl where u is the velocity, g the acceleration of free fall and l a characteristic length (in nautical engineering, the hull length) which, in this application to terrestrial locomotion, is the height h of the hip from the ground. The Froude number then becomes u^2/gh.

Considerations of physical similarity predict that the movements of animals of geometrically similar form but of different sizes will be geometrically similar only when they move with the same Froude number u^2/gh (that is, when the squares of their speeds are proportional to their linear dimensions). Geometrically similar movements require equal values of λ/h (that is, their stride lengths also must be proportional to their linear dimensions). The theory of physical similarity further predicts that, even when the Froude numbers are not the same, λ/h will be a function of the Froude number, although this function is not defined; we are obliged to obtain this relationship by empirical methods. It should be noted that stride length λ in this paper means the distance between corresponding points on successive prints of the same foot[3]; the term is used to mean half this distance in some accounts of dinosaur tracks.

Animals of different sizes are not geometrically similar; there seems, however, to be a rather widespread relationship between λ/h and u^2/gh (ref. 4). Figure 1*a* shows that for mammals as diverse as jirds, men and horses

$$\lambda/h \simeq 2.3(u^2/gh)^{0.3} \qquad (1)$$

Figure 1*b* shows that for man, and presumably for other animals, the relationship between stride length and speed is not appreciably different for firm ground and soft mud. Equation (1) can be written

$$u \simeq 0.25 g^{0.5} \lambda^{1.67} h^{-1.17} \qquad (2)$$

so that u can be estimated from known values of λ and h. λ can be measured accurately from tracks but h can only be estimated from the size of the footprints (see below). Equation 2 shows that if h is overestimated by 10%, u will be underestimated by 11%.

The tracks to be considered were made by unidentified bipedal dinosaurs and sauropods. It is assumed in each case that the metatarsophalangeal joint rested on the ground, but the tarsometatarsal joint did not. If this is correct, the length of the hind footprint is 0.23–0.28h for many bipedal dinosaurs of a wide range of sizes, both Theropoda and Ornithopoda, and about 0.25h for *Apatosaurus* (Sauropoda) (see for instance the photographs of skeletons in refs 5 and 6). It will therefore be assumed that hind footprint length is 0.25h in all cases.

All the data in Table 1 refer to trails of fairly evenly spaced footprints, indicating progression at fairly constant speed. The plan in ref. 7 from which some of the data are taken has

Fig. 1 Graphs on logarithmic coordinates of relative stride length (λ/h) against Froude number (u^2/gh). *a*, Data from travelling on firm ground or treadmills for men[10,11] walking and running, horses[12], jirds (*Meriones unguiculatus*, unpublished observations of S. N. G. Frodsham and R. McN. A.), elephant[12] (*Elephas maximus*) (●) and ostrich[12] (*Struthio camelus*) (○). *b*, New data for man walking and running on hard ground (○), sandy mud (⊙) and soft mud (●) at Snettisham beach, Norfolk. The subjects were an adult man and, two children (aged 11 and 13), each of whom made 4–8 walks or runs at various speeds (in random order) over a 25 m course on each substrate. They made moderate footprints in the sandy mud and very deep ones in the soft mud, on which it was very difficult to run. The broken line on each graph is

$$\lambda/h = 2.3(u^2/gh)^{0.3}.$$

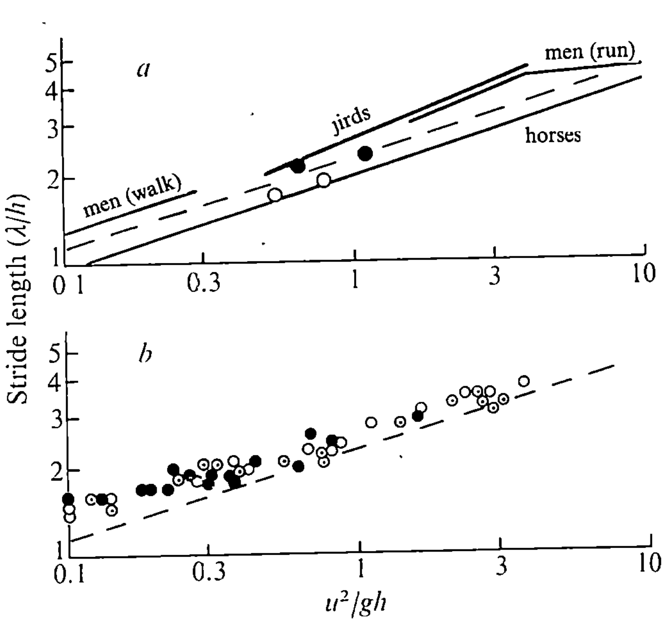

Table 1 Dimensions of dinosaur tracks and data estimated from them

	Bipedal dinosaurs						Sauropods	
Reference	13	7	7	14	1	1	7	7
Stride length, λ (m)	3.0	3.0	2.4	1.3	1.8 –2.5	1.5 –1.8	2.5	1.6
Hind foot length (m)	0.53	0.50	0.24	0.27*	0.28–0.35	0.15–0.20	0.76	0.38
Hip height, h (m)	2.1	2.0	1.0	1.1	1.1 –1.4	0.6 –0.8	3.0	1.5
Relative stride length, λ/h	1.4	1.5	2.5	1.2	1.7	2.4	0.8	1.1
Speed, u (m s^{-1})	2.0	2.2	3.6	1.2	2.2	2.9	1.0	1.1

*Footprint length taken from measurements at the British Museum (Natural History). Other dimensions are taken from the published descriptions.

no scale but the paper includes a photograph of part of the same area marked out in yard squares, from which the scale has been determined. The speeds are rather low, especially those of the sauropods. Large and small sauropods seem to have been walking at the same speed, consistent with the suggestion that they were travelling as a herd[1,7].

Mammals generally change from a walk to a run or trot when the Froude number reaches about 0.6 and λ/h reaches 2.0 (ref. 4). Of the tracks recorded in Table 1, only the two fastest seem to show running, and both were made by fairly small dinosaurs. This does not necessarily mean that large dinosaurs never ran, but it seems to conform better with the traditional image of lumbering dinosaurs than with that of the lively runners shown in some recent restorations[8,9].

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Stereoscopic vision within the schizochroal eye of trilobites

Most trilobites had holochroal eyes which were probably analogous with the compound eyes of most living arthropods[1]. Trilobites of the suborder Phacopina had schizochroal eyes, in which comparatively few large separate lenses are distributed over the eye surface. Clarkson and Levi-Setti[2] showed that the schizochroal eye is unique in structure, in that each lens is biconvex and is made of two calcitic elements of different refractive indices, separated by an aspherical surface with a configuration such that the lens was corrected for spherical aberration. A modified interpretation by Campbell[3] suggests that the e and o rays were transmitted through the calcite lens to focus in the same plane. Since chromatic aberration is not important in seawater more than a few metres deep, either interpretation implies that each lens of the schizochroal eye was capable of forming an accurate image of its field of vision[2,3].

We suggest that the schizochroal eye was adapted to give stereoscopic vision by the comparison and integration of optically accurate images from lenses within the same eye. Stereoscopic vision depends on the simultaneous perception of the same field of view through two lenses set at some distance apart. The images produced by the lenses should be of sufficient optical quality to define objects at useful resolution, the images should overlap considerably and there should be appropriate neural structures to interpret the information provided by the overlapping images. Towe[4], Clarkson and Levi-Setti[2] and Campbell[3] have already established the optical quality of the lenses. It remains to be shown that there would have been useful overlap between the images produced by adjacent lenses, and that it is reasonable to infer the existence of a suitable neural network behind the eye.

Clarkson[5,6] has measured the angular separation between the axes of the lens on many schizochroal eyes. The vertical spacing between lens axes is typically 2–10°, whereas the horizontal spacing is typically 7–20°. Since no part of the visual field is left unscanned, the cone of useful vision of a typical schizochroal lens would have been about 15–20° (for comparison, the ommatidia of *Limulus* have a useful range within 10–20° of their optic axes[7]).

The approximate hexagonal packing of lenses on the schizochroal eye usually included files of lenses arranged dorso-ventrally Coupled with the astigmatism of the eye surface, this meant that the dorso–ventral files of lens axes formed "visual strips"[5]—files of lens axes with low angular separation, running nearly vertically across the visual field, each file of lenses separated by a significant angle from the next file. If each lens had an effective visual field of 15–20°, the visual fields of neighbouring lenses on each visual strip would have overlapped greatly, whereas the visual field of any given lens would only have overlapped marginally with its nearest neighbour on the next visual strip. Thus, given appropriate neural integration, the potential for stereoscopic vision was very great within each visual strip, but must have been marginal between strips.

We suggest that the large horizontal spacing between visual strips was such as to ensure that the whole visual field of the eye was scanned adequately, while the close vertical separation between lens axes along the visual strips allowed stereoscopic scrutiny over the whole visual field. Some phacopids had eyes in which the angular separation between lenses on visual strips approached the separation between strips. The arguments developed here would comfortably include these species, since the angles involved are never more than 6–7° (ref. 6), and would have permitted stereoscopic integration between lenses on the strips.

Rudwick and Clarkson[5] have shown how a trilobite might have detected an approaching object as it appeared successively on images produced by different lenses. In the ideal case, any array of closely spaced lens axes—as in a holochroal eye—would have been able to detect approaching objects with equal sensitivity. But in the real world of the trilobite, stereoscopic scrutiny by cooperating lenses arranged in files would more accurately have detected the existence, size, speed and character of objects in the field of view.

Stockton and Cowen (unpublished) have constructed a simple neurophysiological model of the integration of visual images within a linear array of lenses. According to this model, it is reasonable to suppose that a trilobite with schizochroal eyes could have had the neural apparatus to perceive objects stereoscopically. The model includes predictions of features of morphology found among phacopid trilobites, and it is amenable for testing by further study of the optics of phacopid lenses.

Phacopid trilobites had visual fields that usually extended through 360° in the horizontal plane, but only from 0 to 40° at most above the horizontal. If our suggestion is correct, they would have had stereoscopic vision throughout that field. In fact, they would have had a wider range of stereoscopic vision than any organism that has ever lived.

The system was essentially a defensive one. Hunting predators have stereoscopy which is best developed in a limited anterior field. Prey species often have all-round vision, but usually have limited stereoscopic capability. Phacopid trilobites can thus be interpreted as benthic animals with special adaptations for detecting moving enemies on the sea floor in the photic zone. Cephalopods, arthropods and (in the Devonian) fish would have been likely predators on phacopids.

The Phacopina became extinct at the end of the Devonian period, long before trilobites as a class became extinct. Although less than 10% of Ordovician trilobite genera were Phacopina, however, their representation rose to 10–20% during the Silurian and reached 20–40% during the Devonian[8]. Trilobites with schizochroal eyes were relatively successful for 150 Myr. Nothing like the schizochroal eye has ever re-evolved among the hundreds of thousands of species of later arthropods.

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Parent–offspring resemblance for specific cognitive abilities in two ethnic groups

A LARGE scale study of familial factors in mental ability is in progress in Hawaii and data are being obtained on 15 cognitive variables (Table 1), various environmental indices, and blood group and enzyme systems. After three years of testing, there are now sufficient data (1,490 families; 5,077 individuals) for preliminary genetic analyses. Measures of parent–offspring resemblance for members of the two largest ethnic groups in our sample, Americans of European ancestry (AEA; 739 families) and Americans of Japanese ancestry (AJA; 244 families), are presented here. These measures provide upper-bound estimates for the heritability of performance on the individual tests and factor scores[1,2], in addition to a test of the hypothesis[3,4] that spatial visualisation ability is influenced by a sex-linked major gene. Relatively small scale studies of parent–offspring resemblance for specific cognitive abilities have been reported for a Caucasian sample[5] and a Korean sample[6]; however, to the best of our knowledge, this is the first comparative study of parent–offspring resemblance for measures of cognitive abilities in two ethnic groups.

A 'family', for our purposes, consists of both biological parents, 60 yr of age or younger, and one or more of their offspring, 13 yr of age or older. Because of the presence of marked age effects[2], age correction was deemed necessary; such correction was accomplished by using a z-score banding technique. Age bands consisted of yearly intervals for the ages 15–20 and 34–55, and of 2–6-yr intervals for other ages (depending on sample size).

In the absence of an environmental correlation between relatives, the regression of offspring's score on mid-parental score provides a direct estimate of heritability in its "narrow sense", that is, the proportion of the observed phenotypic variance due to the average effects of the genes[7]. This method for estimating heritability has several advantages over other methods, including its insensitivity to the effects of non-random mating. (Assortative marriage for specific cognitive abilities in this study has been discussed elsewhere[8].) For at least some measures of human mental ability, however, environmental correlations between family members are likely to be non-zero and positive. The regressions of offspring on mid-parental values presented here should, therefore, be regarded as measures of phenotypic (genetic and/or environmental) similarity and only upper-bound estimates of heritability.

The regression coefficients for each cognitive test and for five factor scores are presented in Table 2. Two sets of coefficients are given, one uncorrected and one corrected for test reliability. It is important to be clear about when and when not to adjust for lack of perfect test reliability[9]: when estimates are to be used in prediction equations with individual scores (for example, to predict response to selection), uncorrected estimates must be used, but when estimates obtained from tests with different reliabilities are to be compared, correction is appropriate. Since considerable variation can be seen among the reliabilities presented in Table 1, only the corrected regression coefficients will be examined for evidence of differential heritability.

Standard errors for the corrected regression coefficients in Table 2 are sufficiently small, especially for AEA, to indicate that at least some of the variability among estimates is real. An indication of the reliability of the differential estimates among tests is the marked congruence between ethnic groups. The correlation between AEA and AJA coefficients across the 15 individual tests is highly significant (Spearman rank correlation = 0.77, $P < 0.01$). With regard to the four varimax rotated factor scores, the regressions are moderately high for verbal and spatial ability, but somewhat lower for visual memory and perceptual speed in both ethnic groups. These results are highly consistent with previous evidence[10] for the differential heritability of specific cognitive abilities (especially for verbal ability and memory) and afford an opportunity for identification of those environmental factors that differentially affect cognitive abilities.

Our tests also provide a measure of 'general intelligence'. The progressive matrices test is a modified form of the well known test of abstract reasoning developed by Raven[11], a "culture-fair" test which is regarded by many as being one of the best single measures of general intelligence. Our modified form is reduced in length to 30 items (20-min time limit in group administration), but retains a relatively high reliability (see Table 1). Regression of offspring on mid-parental value (uncorrected) for this test is 0.51 for AEA subjects and 0.27 for AJA. These results are consistent with those of Guttman[12], who found the correlation between child and mid-parent in a sample of 100 families in Israel to be 0.41 for the complete test.

The unrotated first-principal component score may also be used as a measure of general intelligence. The regressions of offspring on mid-parental values (uncorrected) for this measure

Table 1 Cognitive tests, test times and reliabilities for two ethnic groups: Americans of European ancestry and Americans of Japanese ancestry

Test	Cognitive factor*	Test time	Reliability†
Primary mental abilities (PMA) vocabulary	V	3 min	0.96
Visual memory (immediate)	M	1-min exposure/1-min recall	0.58
Things (a fluency test)	V	2 parts/3 min each	0 74
Sheppard–Metzler mental rotations (modified for group testing by Vandenberg)	S	10 min	0.88
Subtraction and multiplication	P	2 parts/2 min each	0.96
Elithorn mazes ("lines and dots"), shortened form	S	5 min	0.89
Educational testing service (ETS) word beginnings and endings	V	2 parts/3 min each	0.71
ETS card rotations	S	2 parts/3 min each	0.88
Visual memory (delayed recall)	M	1 min	0.62
PMA pedigree (a reasoning test)	V,S,P	4 min	0.72
ETS hidden patterns	S	2 parts/2 min each	0.92
Paper form board	S	3 min	0.84
ETS number comparisons	P	2 parts/1.5 min each	0.81
Whiteman test of social perception	V	10 min	0.69
Raven's progressive matrices, modified form	S	20 min	0.86

*Varimax rotated factor loading $\geqslant 0.40$ (V, verbal factor; S, spatial factor; P, perceptual speed factor; M, visual memory factor).
†From Wilson *et al.*[2] Details concerning the testing procedure and factor analyses are available in this source.

are 0.61 and 0.42 for AEA and AJA, respectively. These values are comparable with those obtained by Williams[5], who reported that the regression of offspring on mid-parent was 0.46 for total IQ as measured by the Wechsler Intelligence Scales. Since the regression of offspring on mid-parent may only be regarded as an upper-bound estimate of heritability, these results suggest that the narrow-sense heritability for tests of general intelligence may be lower than the value of 0.71 reported by Jinks and Fulker[13] in their re-analysis of previously published data.

Scarr-Salapatek[14] concluded from twin data that the heritability of scholastic aptitudes may differ in different racial groups as a function of socioeconomic status. Although her conclusions have been criticised[15] on the basis of statistical considerations, she has recently[16] reported additional evidence for such a difference. Even though the educational and occupational levels of the two ethnic groups included in the present study are roughly comparable, differences in parent–offspring resemblance may exist between groups. From Table 2 it can be seen that 14 of the 15 regression coefficients for the individual tests are higher for AEA subjects. Only two of these differences however, are significant. When these data were examined for differences in parent–offspring resemblance as a function of socioeconomic status within ethnic groups, no significant differences were observed.

Single-parent, single-child correlations were also estimated for the individual tests and factor scores in order to explore the possibility of sex-linked inheritance. Stafford[3] first reported a pattern of parent–offspring correlations which suggested that spatial visualisation ability may be influenced by a sex-linked major gene. The expected pattern of correlations (r) for such a character is as follows

$$r_{\mathrm{FS}} < r_{\mathrm{MD}} < r_{\mathrm{MS}} = r_{\mathrm{FD}}$$

where F, M, S and D are father, mother, son and daughter, respectively. Stafford's original finding has been given considerable support by the more recent report of Bock and

Table 2 Regression of mid-child on mid-parent (± s.e.) for cognitive tests and factor scores in two ethnic groups

	Uncorrected		Corrected†	
	AEA	AJA	AEA	AJA
Tests				
Vocabulary	0.65±0.05	0.57±0 07	0.68±0 04	0.59±0.07
Visual memory (immediate)	0.15±0.05	0.16±0.08	0.26±0.05	0.28±0.08
Things	0.41±0.05	0.27±0.09	0.55±0.04	0.36±0.09
Mental rotations	0.42±0.05	0.37±0.09	0.48±0.05	0.42±0.09
Subtraction and multiplication	0.38±0.04	0.29±0.09	0.40±0.04	0.30±0.09
"Lines and dots" (Elithorn mazes)	0.22±0.05	0.19±0.08	0.25±0.05	0.21±0.08
Word beginnings and endings	0.40±0.04	0.39±0.09	0.56±0.04	0.55±0.08
Card rotations*	0.46±0.05	0.25±0.08	0.53±0.04	0.28±0.07
Visual memory (delayed)	0.33±0.05	0.22±0.09	0 53±0.04	0.35±0.09
Pedigrees	0.53±0.04	0.46±0.08	0.74±0.04	0.64±0.07
Hidden patterns	0.43±0.05	0.27±0.08	0.47±0.05	0.29±0.08
Paper form board	0.53±0.04	0.48±0.09	0.63±0.04	0.57±0.09
Number comparisons	0.39±0.05	0.32±0 08	0.48±0.04	0 40±0.08
Social perception	0.27±0.05	0 24±0.07	0.39±0.05	0.35±0.07
Progressive matrices*	0.51±0.04	0.27±0.07	0 59±0.04	0.31±0.07
Factors				
Verbal	0.57±0.04	0.51±0.07	0.65±0.04	0.58±0.07
Spatial	0.57±0.05	0.41±0.08	0.61±0.04	0 44±0.08
Perceptual speed	0.41±0.05	0.29±0.09	0.46±0.04	0.32±0.09
Visual memory	0.32±0.05	0.22±0.09	0.44±0.05	0.31±0 09
First principal component*	0.61±0.04	0 42±0.07	0.63±0.04	0.43±0.06
No. of families‡	739	244	739	244

*Significant difference between AEA and AJA regression coefficients ($P \leqslant 0.05$).
†Corrected for test reliability.
‡AEA data from 482 one-child families, 224 two-child families, 30 three-child families and 3 four-child families. AJA data from 198 one-child families, 36 two-child families, 9 three-child families and 1 four-child family.

Table 3 Single-parent, single-child correlations for cognitive tests and factor scores in two ethnic groups*

	AEA				AJA			
	Father–son	Mother–daughter	Mother–son	Father–daughter	Father–son	Mother–daughter	Mother–son	Father–daughter
Tests								
Vocabulary	0.35	0.37	0.36	0.30	0.35	0.31	0.38	0.19
Visual memory (immediate)	0.05	0.10	0.06	0.08	0.06	0.06	0.06	0.23
Things	0.17	0.25	0.25	0.18	0.24	0.14	0.12	0.16
Mental rotations	0.15	0.32	0.16	0.23	0.30	0.10	0.14	0.34
Subtraction multiplication	0.28	0.25	0.17	0.17	0.18	0.14	0.19	0.04
"Lines and dots" (Elithorn mazes)	0.07	0.17	0.17	0.07	0.08	0.04	0.14	0.19
Word beginnings and endings	0.23	0.27	0.24	0.26	0.20	0.14	0.25	0.22
Card rotations	0.26	0.36	0.19	0.22	0.26	0.09	0.12	0.11
Visual memory (delayed)	0.16	0.10	0.14	0.21	0.18	0.10	0.10	0.11
Pedigrees	0.26	0.38	0.32	0.26	0.23	0.31	0.22	0.28
Hidden patterns	0.21	0.32	0.20	0.27	0.10	0.18	0.12	0.26
Paper form board	0.27	0 36	0.30	0.40	0.24	0.24	0.26	0.21
Number comparisons	0.25	0.27	0.24	0.17	0.25	0.09	0.22	0.36
Social perception	0.11	0.15	0.18	0.12	0.09	0.21	0.07	0.26
Progressive matrices	0.20	0.29	0.38	0.27	0.10	0.17	0.22	0.26
Factors								
Verbal	0.27	0.30	0.40	0.31	0.36	0.29	0.35	0.24
Spatial	0.27	0.41	0.28	0.32	0.33	0.13	0.21	0.31
Perceptual speed	0.28	0.27	0.22	0.18	0.23	0.04	0.22	0.20
Visual memory	0.15	0.13	0.13	0.18	0.12	0.10	0.09	0.20
First principal component	0 29	0.45	0.41	0.36	0.30	0.28	0.29	0.30
No. of pairs	434	438	434	438	130	138	130	138

*Correlations greater than 0.10 are significantly different from zero ($P \leqslant 0.05$) for AEA subjects, whereas those greater than 0.17 are significant for AJA subjects.

Kolakowski[4]. Single-parent, single-child correlations obtained from the data of the present study are presented in Table 3. Of the correlations involving individual tests of spatial ability (mental rotations, "lines and dots", card rotations, hidden patterns, paper form board, progressive matrices), only the set for progressive matrices for AJA subjects conforms to the order expected of a character influenced by a sex-linked major gene. An hypothesis of homogeneity among the four correlations in this set could not be rejected ($\chi^2 = 2.06$, $P \geqslant 0.50$, d.f. $= 3$); thus, results of this analysis do not support the hypothesis that spatial ability is influenced by a sex-linked major gene. Nor is sex linkage indicated by the pattern of correlations for the spatial factor scores. In fact, the highest correlation for AEA families is between mother and daughter and that for AJA families is between father and son. These results suggest that alternatives to the sex-linked, major-gene hypothesis (for example, the sex-modified threshold model[17]) for the inheritance of spatial ability should also be considered.

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Relative role of environmental and genetic factors in respiratory adaptation to high altitude

A VARIETY of adaptive mechanisms which aid in the physiological adjustment to the hypoxic environment of altitude have been reported in man[1]. Although visitors to high altitudes increase ventilation in response to environmental hypoxia, natives in the Andes and Himalayas show a blunted or absent ventilatory response to acute hypoxia both at rest and during exercise[2–5]. Adult high altitude natives in the Andes have larger lungs with a greater gas exchange surface, as judged by physiological tests of lung volume and diffusing capacity, than sea level natives or short term visitors at altitude[6–9]. Whether these respiratory adaptations in man are determined solely by environment or relate to genetic traits is uncertain. The age at which these pulmonary adaptations to hypoxia occur is not known, neither is it certain whether migrants to and from high altitude reversibly acquire these physiological changes. To investigate further the mechanism of pulmonary adaptations to chronic hypoxia and to clarify the position of genetic factors in this process, we undertook an expedition to Peru from April to August 1975. Control of ventilation and lung volumes were studied in different age groups, from neonatal period to adulthood, both at high altitude and sea level. It was found that the blunted ventilatory response to hypoxia and large lung volumes developed gradually after birth over a period of many years, and that the phenomena were at least partly reversible at sea level. The results showed that the adaptive response in respiratory physiology were determined by environmental rather than genetic factors.

The high altitude station was in Puno, a town on Lake Titicaca in the Altiplano (3,850 m; barometric pressure = 484 torr; $P_{\mathrm{IO}_2} = 91$ torr) and the laboratory at low altitude was established in Tacna (800 m; barometric pressure = 715 torr; $P_{\mathrm{IO}_2} = 139$ torr). Subjects were selected from the indigenous population of the two areas and from individuals who had migrated to or from high altitude. They were categorised by genetic background, birth place and parents' birth place, and by length of residence at high altitude. Most of the indigenous population of high altitude and migrants from high altitude were Peruvian Indians and those from sea level were mestizos (mixed extraction). Subjects ranged in age from several hours after birth to 50 yr.

The following measurements were made and recorded continuously using a Honeywell ultraviolet polygraph: inspired ventilation by a Fleisch pneumotachograph with integrating circuit; arterial O_2 saturation by Waters Oximeter; end-tidal P_{O_2} with a Beckman O_2 analyser; and P_{CO_2} with Godart capnograph. Neonates and infants were tested using a tight-fitting face mask with minimal dead space while the subjects were asleep in a thermoneutral condition. Children and adults were tested using a mouthpiece and nose clip while they were awake and seated in a chair. Ventilatory sensitivity to hypoxia was measured by both transient and quasi-steady-state techniques[10]. The sensitivity was expressed as the ratio between percentage increase in ventilation and percentage decrease in arterial O_2 saturation. A ratio < 1 indicated blunted hypoxic sensitivity; a ratio of up to 5 indicated intermediate response and > 5 a normal response. Ventilatory response to hypercapnia was studied by the conventional rebreathing technique. Lung volumes were measured by recording the largest of three vital capacity manoeuvres in children and adults, and crying vital capacity was measured in neonates and infants[11].

In 20 full-term highlander neonates < 5 d old, raising the inspired oxygen concentration from ambient (20.9%) to > 60% produced two types of response; an immediate response which was a small decrease in ventilation, followed by a sustained increase during prolonged oxygen breathing. Lowering the inspired oxygen concentration to 12–15% caused a small transient increase in ventilation and then a depression, which was promptly reversed by raising P_{IO_2}. The pattern of responses to alterations in inspired oxygen was similar in 35 full-term neonates studied at a low altitude. All the subjects, however, showed increased ventilation during rebreathing CO_2 in O_2. In infants up to the age of 6 months studied at both high and low altitude, lowering the inspired oxygen concentration produced a sustained increase in minute ventilation which promptly decreased on increasing P_{IO_2}. Thus, hypoxic ventilatory drive matures after birth at high altitude as it does at sea level[12].

Except for one 6-yr-old girl and a boy all 43 children up to the age of 12 yr showed an increase in ventilation with acute hypoxia and a decrease with hyperoxia at high altitude. These responses were similar to those of children studied at low altitude, excluding the two exceptions.

Of the 58 high altitude natives between 13 and 20 yr, ~ 30 showed a normal response, 16 an intermediate response, and 10 a blunted response to hypoxia. Acute hyperoxia depressed ventilation in those subjects who responded well to hypoxia. All 40 adult high altitude natives over the age of 22 demonstrated a blunted ventilatory response to hypoxia. Blunted responses occurred independent of the individual's parentage and genetic background. These results showed that a normal hypoxic ventilatory drive was developed in the natives of high altitude before it was substantially lost during adult life. The hypercapnic response in the adults was near normal.

Table 1 Respiratory adaptation to high altitude

Duration of hypoxia	Ventilatory response to acute hypoxia	Increased vital capacity from predicted sea level values
Birth to 1 week	±	0
2–6 months	+++	0
5–8 yr	++++	+
8–12 yr	+++	++
12–20 yr	++	+++
20–25 yr	+	+++

None of the migrants to high altitude demonstrated a diminished ventilatory response to hypoxia, although the longest period of residence at high altitude in migrants was 6 yr. All but one of the 28 migrants from high altitude to sea level who were less than 12 yr old showed normal ventilatory response to acute hypoxia. Recent migrants more than 15 yr old showed a diminished ventilatory response to hypoxia. These results conform with the findings at high altitude. Adult migrants who had been at sea level for long periods, however, usually showed a normal response to hypoxia, suggesting that the acquired blunted hypoxic response was a reversible phenomenon. Loss and reversal occurred over a prolonged period.

Vital capacity expressed in absolute terms or as ml per kg body weight was similar in 12 high altitude native neonates and in 20 sea level neonates, and was generally within the range of normal predicted values for crying vital capacity data[11]. Crying vital capacity tended to be similar in low and high altitude infants less than 1 yr old. High altitude children between 9 and 13 yr old had vital capacities that averaged 2.87±0.62 l, or 121±13.7% of predicted values based on age and height. The accelerated lung growth curve persisted throughout the teenage years, so that native young adults 18–21 yr old had vital capacities which were 5.05±0.67 l, or 145±16.7% of predicted, whereas vital capacity of sea level controls was 3.73±0.31 l, or 104±8.2% of predicted. The increase above predicted values of vital capacity in highlanders occurred independently of genetic background and place of birth of the parents. Thus, the normal vital capacity that occurs with age was greater at high altitude than at sea level.

In general, individuals of any age who had migrated to high altitude tended to have normal or near normal vital capacities if their stay at altitude was < 3 yr. Those migrants (ages 7, 9, 10 and 18 yr) who had lived at high altitude for more than 4 yr had vital capacities considerably above predicted values.

Thus the ventilatory response to hypoxia and vital capacity are similar in neonates and infants at sea level and at high altitude. Vital capacity tends to increase in the postnatal period and continues to do so throughout the teenage period. The blunted hypoxic response, however, only begins to manifest itself in teenage life, becoming universal in adults.

Our results, which are summarised in Table 1, suggest that the characteristic diminished ventilatory response to hypoxia and large lung volumes found in adult high altitude natives are determined environmentally, rather than genetically. A genetic trait tends to be excluded on the grounds that offspring of lowlanders born and bred at high altitude showed the same phenomena as native highlanders. A similar developmental adaptation of improved physical performance capacity in response to hypoxia has been reported[13].

The similarity in the lack of a sustained hypoxic ventilatory drive in sleeping neonates at sea level and high altitude suggests that the normal hypoxic control of ventilation develops fully only after birth. A reflex hypoxic drive is thus of limited value for survival in newborns at high altitude. In addition, newborns at low and high altitude have similar ventilatory control mechanisms and lung volumes, suggesting that no genetic or intrauterine adaptive changes occur in these parameters in preparation for life at high altitude. Appropriately, however, an increase in placental surface area relative to the body weight of the foetus occurs at high altitude[14]. It is presumably only after birth that high altitude neonates are exposed to a greater hypoxic stimuli than at sea level. The adult pattern of responses and adaptations to hypoxia begins to develop shortly after birth. In teenage life a variety of effective gas transport adaptations develop, such as the increased area for gas exchange provided by large lungs, and ventilatory hypoxic drive assumes a less important role.

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Elimination of aziridine residues from chemosterilised mosquitoes

CRITICISM[1–4] of the use of the alkylating agent thiotepa (triethylene thiophosphoramide) to sterilise mosquitoes, as part of an eradication programme in India, focused on its toxicity and the possibility that it or its breakdown products would harm other animal components of the food chain. For example, a Canadian team found reduced fertility in spiders fed on mosquitoes sterilised with thiotepa[5]. Some means was needed to ensure that no aziridine residues—breakdown products of thiotepa—remain in the insects after release into the environment. We have now achieved this, essentially by dipping the chemosterilised insects into acid and then alkaline solutions.

Complete breakdown of 3.1% thiotepa solution has been reported to occur in 90 min at *p*H 4, but aziridine activity persists for 24 h. This is due to the production of ethyleneimine which is stable in acid medium and tends to accumulate. Ethyleneimine is cytotoxic and mutagenic and may in some cases be carcinogenic[6] and therefore potentially hazardous. It breaks down to non-aziridine compounds at *p*H 9 (ref. 7).

The decomposition of thiotepa in acid medium was monitored by bioassay. A 0.6% solution was prepared in 0.01 N H_2SO_4, *p*H 1.8–2.0, and *p*H 3 was maintained by addition of small increments of N H_2SO_4 for 90 min. A hundred mosquito pupae (*Culex pipiens fatigans*) were placed in this solution for 3 h, washed twice in tap water and held in cages at 28–29 °C and 75% relative humidity until emergence. An equal number of virgin females was introduced into the cages and egg rafts were collected for routine bioassay. Fifty rafts, scored for percentage hatch, gave 92.7% fertility, whereas the controls dipped in water and in 0.6% thiotepa (*p*H 8) showed 94.9% and 0.01% fertility, respectively. Breakdown of thiotepa was complete in acid solution and the main decomposition product, ethyleneimine, proved inactive as a sterilant for *C. p. fatigans*. Therefore dipping sterilised pupae in acid solution (0.0025 N H_2SO_4, *p*H 2.6–2.8) for 90 min would ensure complete decomposition of thiotepa to ethyleneimine. Although no appreciable accumulation of ethyleneimine is likely, traces present as the intermediate decomposition product in sterile males may contaminate the environment. Ethyleneimine is hydrolysed to deuteriophosphoric acid and D_2NCH_2-CH_2OD, and this conversion can be accelerated by dipping acidified pupae in alkaline solution, *p*H 9 (ref. 7), thus making the mosquitoes free of any traces of aziridine compounds.

We tested the biological fitness of sterile male *C. p. fatigans* produced when pupae were dipped in 0.6% thiotepa, *p*H 8, for 3 h, washed twice in tap water, dipped in 0.0025 N H_2SO_4 for 90 min, washed in tap water, dipped in alkaline solution, *p*H 9.1–9.4, for 90 min, washed and transferred to tap water for emergence. No pupae died after this treatment. To assess survival, 100 sterile and 100 normal males were held in cages (ten replicates) and offered water-soaked raisins daily. The number of mosquitoes found dead in each cage was recorded at 24-h intervals. Weekly (%) mortality of sterile males averaged 1.9, 12.7 and 17.4 (cumulative total 32.2 in 3 weeks) and controls averaged 3.6, 13.2 and 20.3 (cumulative total 37.1 in 3 weeks). Thus the treatment had no effect on the longevity of the mosquitoes.

Sterile males were also tested for mating competitiveness. In ten laboratory cages, 100 sterile and 100 normal, 1-2-d-old males and 100 virgin, 2-d-old females were fed on raisins and allowed to mate for 4 d. They were offered pigeons for a blood meal. A total of 622 egg rafts was collected, of which 327 were sterile. As sterility averaged 52.57%, sterile males proved competitive.

Thus residues of thiotepa, and other aziridine compounds can be eliminated from sterile mosquitoes by dipping them in acid and alkaline solutions. Residue-free emergent adults survive well for at least 3 weeks. As sterile males compete favourably with normal males in the insemination of females, mass release of these sterile males for population control would not be hazardous to the food chain and would not contaminate the environment directly or indirectly.

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Augmentation of human monocyte chemotactic response by levamisole

THE antihelminthic drug, levamisole ((−)-tetramisole) has been shown to possess potent immunostimulatory properties as demonstrated by its ability to enhance delayed hypersensitivity responses in aged individuals and in patients with cancer[1,2]. In addition, animals treated with (−)-tetramisole have heightened activity of their reticuloendothelial systems as measured by clearance of carbon particles and phagocytosis of sensitised sheep erythrocytes[3,4]. Although the mechanism of action of levamisole remains obscure, its pharmacological properties suggest that it may affect macrophage function and as such could be a useful tool to probe the pathophysiology of this cell. This report describes the effect of (−)-tetramisole and its isomers on monocyte chemotaxis *in vitro*, and demonstrates a potential mechanism of action for these drugs as well as their usefulness for delineating mechanisms of leukocyte chemotactic responsiveness.

Human monocyte chemotaxis was assayed *in vitro* by a minor modification of a previously described technique[5]. Briefly, peripheral blood monocytes were isolated on Ficoll–Hypaque gradients, washed extensively, and resuspended to contain 1.5×10^6 monocytes ml^{-1} in medium RPMI 1640 (Grand Island Biological Company, Grand Island, New York). Cell suspension (0.4 ml) was placed in the upper compartment of a modified Boyden chamber and separated from the chemotactic or control stimulant in the lower compartment by a 5.0-μm polycarbonate (Nuclepore) filter. After incubation for various times at 37 °C in humidified air, the chambers were emptied, the filters removed, fixed in ethanol and stained with haematoxylin. Chemotactic response was assessed by counting and averaging the number of cells which migrated completely through the filter per microscopic field in 20 oil immersion fields (×1,540). All assays were carried out in triplicate, each experiment repeated at least twice with cells from different donors and a single representative experiment presented.

Human polymorphonuclear leukocyte (PMN) chemotaxis was quantified similarly[6]. PMNs were isolated from whole blood by sedimentation with 3% Dextran in saline, washed extensively, and resuspended to 2.2×10^6 PMN ml^{-1} in Gey's balanced salt solution (BSS) containing 2% bovalbumin (Flow Laboratories, Rockville, Maryland), *p*H 7.2. A 5.0-μm nitrocellulose filter (Millipore) was used to separate the upper and lower compartments of the chemotaxis chambers. After incubation for 3 h at 37 °C, the filters were processed as described previously[6]. Chemotactic response was expressed as the average number of PMNs migrating completely through the filter per field in 20 high power fields (×780).

To determine the effect of (−)-tetramisole, its stereoisomer, (+)-tetramisole, and *p*-Br-(−)-tetramisole (Janssen, Beerse, Belgium) on monocyte chemotaxis, the isolated cells were incubated with various doses of the drugs contained in RPMI 1640 or with medium alone, and tested for chemotactic response to lymphocyte-derived chemotactic factor (LDCF), endotoxin-activated human serum (AHS) or to the chemotactically active peptide, *N*-formyl-methionyl-phenylalanine (f-met-phe)[7]. Table 1 illustrates that doses of (−)-tetramisole ranging from 10^{-5}–10^{-3} M enhanced the chemotactic response of monocytes to the three chemotactic factors by 20–148%. Similar doses of (+)-tetramisole had no significant ($P > 0.2$) effect on monocyte chemotactic responsiveness to LDCF. Cells incubated with *p*-Br-(−)-tetramisole (10^{-6}–5×10^{-5} M) also exhibited an increased chemotactic response to LDCF; however at doses higher than 5×10^{-4} M, *p*-Br-(−)-tetramisole depressed chemotaxis and was toxic to the monocytes as judged by Trypan blue exclusion. The effect of (−)-tetramisole was removed by washing the cells extensively after a 30-min incubation with the drug. Enhanced monocyte chemotactic responsiveness was not seen when (−)-tetramisole was placed in the lower compartment of the chemotaxis chamber and incubated with the chemotactic factors rather than with the cells. These data indicated that (−)-tetramisole exerts its effect primarily on the monocyte, and that enhancement of monocyte chemotactic responsiveness by (−)-tetramisole is stereospecific.

To determine whether enhancement of monocyte chemotaxis by (−)-tetramisole required binding of the drug to the monocytes, cells were incubated with medium containing various doses of (−)-tetramisole alone or with medium containing (−)-tetramisole plus (+)-tetramisole, and tested for chemotactic response to LDCF. Figure 1 illustrates that doses of 10^{-4} and 10^{-3} M (+)-tetramisole reduced the enhancing effect of (−)-tetramisole. The parallel nature of the curves indicated that (+)-tetramisole inhibited the enhancing activity produced by (−)-tetramisole by competitive binding (k_D for (−)-tetramisole$=1 \times 10^{-4}$; k_D for (+)-tetramisole$=0.8 \times 10^{-4}$). To ascertain whether (−)-tetramisole increased the rate of cell migration or the total number of cells capable of responding to a given dose of chemotactic factor, monocytes were incubated (37 °C for 30 min) with RPMI containing 10^{-3} M (−)-tetramisole or with medium alone, and the number of cells responding to LDCF determined after various times of incubation in the chemotaxis chambers. Figure 2 illustrates that (−)-tetramisole increased both the rate of monocyte chemotactic responsiveness and the maximum number of cells responding chemotactically. .

We next determined whether (−)-tetramisole exerted an effect on another type of cell capable of responding chemotactically. Human PMNs were incubated with Gey's BSS

Table 1 Effect of isomers of tetramisole on human monocyte chemotactic response

Monocytes incubated with*	Chemotactic activity† of monocytes in response to: LDCF‡	AHS‖	f-met-phe¶
Medium alone	43.9±3.6	79.5±6.3	36.0±3.0
(−)-Tetramisole			
1×10^{-3} M	98.0±1.0	114.8±7.3	89.4±1.7
1×10^{-4} M	73.9±6.9	107.6±10.2	53.1±4.3
1×10^{-5} M	59.1±0.3	95.4±1.3	44.9±5.3
p-Br-(+)-tetramisole			
5×10^{-5} M	82.5±6.8	—	—
1×10^{-5} M	61.7±3.5	—	—
1×10^{-6} M	49.8±2.1	—	—
(+)-Tetramisole			
1×10^{-3} M	49.8±5.3	—	—
1×10^{-4} M	49.2±2.0	—	—
1×10^{-5} M	45.5±5.6	—	—

*Human monocytes were isolated on Ficoll–Hypaque gradients, washed extensively, and resuspended (1.5×10^6 ml^{-1}) in medium containing various molar concentrations of the indicated isomers of tetramisole or in medium alone. After incubation (37 °C for 30 min) the monocytes were placed in the upper compartment of a modified Boyden chamber and tested for chemotactic response.

†Chemotactic activity is expressed as the average number of monocytes in triplicate samples migrating completely through a 5.0 μm polycarbonate filter per oil immersion field (×1,540) ± s.e.m.

‡Lymphocyte derived chemotactic factor (LDCF) was obtained from supernatants of concanavalin A-stimulated lymphocyte cultures. LDCF was diluted to 15% v/v in RPMI 1640 for use as a chemotactic stimulant.

‖Activated human serum (AHS) was prepared by incubation of normal serum with *S. typhosa* endotoxin (1 mg ml^{-1} serum) for 60 min at 37 °C followed by 56 °C for 30 min. AHS was then diluted to 0.5% v/v in RPMI 1640 for use as a chemotactic stimulant.

¶f-met-phe was diluted to 10^{-6} M ml^{-1} RPMI 1640 for use as a chemotactic stimulant.

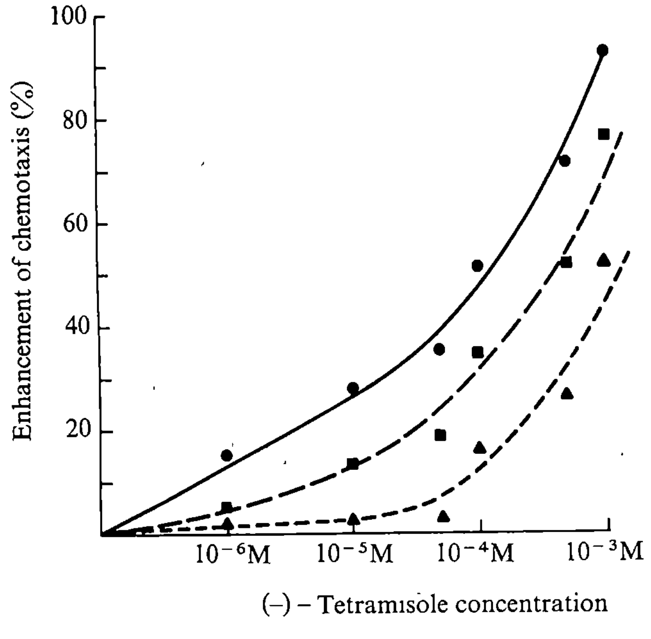
containing 10^{-3}–10^{-6} M (—)-tetramisole or with medium alone and tested for chemotactic responsiveness to various doses of AHS or to f-met-phe. At the doses tested, (—)-tetramisole had no effect on PMN chemotactic response to the two chemotactic factors, indicating a differential effectiveness of the drug for monocytes.

The above results demonstrate that (—)-tetramisole and *p*-Br-(—)-tetramisole exert stimulatory effects on human monocyte chemotactic response by increasing both the rate of cell migration and the total number of cells capable of responding to a given dose of chemotactic factor. In addition, it seems that the enhancing effect is stereospecific and that the drug must bind to the monocytes since addition of the dextroisomer competitively inhibited the enhancing effect of (—)-tetramisole. Both isomers have approximately the same dissociation constant for monocyte binding ($k_D \sim 10^{-4}$).

Our data demonstrate that (—)-tetramisole stimulates the unidirectional migration of monocytes in response to a given number of chemotactically active molecules. The ability of (—)-tetramisole to enhance monocyte chemotactic function might be one mechanism by which it augments immunological response *in vivo* The mechanism of action of (—)-tetramisole on human monocyte chemotaxis has not yet been determined but it could affect binding of chemotactic factors to the monocyte or the translation of the chemotactic factor–monocyte interaction into a directional cell movement. Little information concerning the mechanism by which chemotactic factors induce unidirectional leukocyte migration is available. The stereospecificity as well as cell-type specificity of the effect of (—)-tetramisole indicate that this drug has differential cell membrane effects on chemotactically responsive cells. This drug and its isomers can therefore be useful tools in delineating mechanisms of unidirectional cell migration.

Fig. 1 Competitive inhibition of the enhancing effect of (–)-tetramisole on monocyte chemotactic response to (+)-tetramisole. Isolated monocytes were incubated (37 °C for 30 min) with medium containing 10^{-3}–10^{-6} M (–)-tetramisole alone (●), or with 10^{-3}–10^{-6} M (–)-tetramisole plus 10^{-3} M (▲) or 10^{-4} M (+)-tetramisole (■), and tested for chemotactic responsiveness to LDCF (15% v/v). k_D for (–)-tetramisole = 10^{-4}; k_D for (+)-tetramisole = 0.8×10^{-4}. % Enhancement =

$$\left(\frac{\text{Chemotactic responsiveness of cells incubated with drugs}}{\text{Chemotactic responsiveness of cells incubated with medium alone}} - 1\right) \times 100.$$



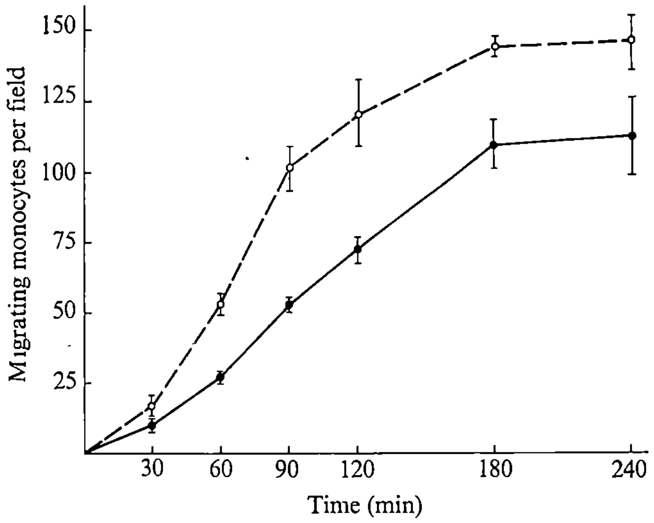


Fig. 2 Kinetics of enhancement of monocyte chemotactic response to (–)-tetramisole. Isolated monocytes were incubated (37 °C for 30 min) with medium alone (●) or containing $10^{-}\mathrm{M}^{3}$ (–)-tetramisole (○), and tested for chemotactic response to LDCF (15% v/v) after the indicated incubation time in the chemotaxis chamber. Vertical bars indicate s.e.m. for triplicate samples.

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A new differentiation antigen defining a subpopulation of mouse T cells

IT is generally accepted that lymphocyte reactions such as the mitogenic response to concanavalin A (con A) and phytohaemagglutinin (PHA), the graft *versus* host (G*v*H) reaction, the helper effect in the antibody response and cell-mediated cytotoxicity (CMC) all involve the activity of thymus-derived lymphocytes (T cells). Because of the paucity of techniques for identifying cells with different functions, however, very little is known about the identity and relationships of T cells involved in these reactions.

In the mouse, a convenient way of identifying functionally distinct subpopulations of T cells has been described by the demonstration that Ly antigens can be used to distinguish between such subpopulations[1,2]. Here we describe an antigenic marker that enables the distinction between cytotoxic ('killer') T cells from T cells involved in the above reactions. Following examples of other previously described differentiation antigens demonstrated by heteroantisera (that is, MBLA[3], MSPCA[4] and MSLA[5]) we have tentatively

Table 1 Suppression of CMC function of spleen cells (SPC) and non-adherent peritoneal cells (NAPC) from H–2-primed donors by pretreatment with Ra-*nu*/*nu* and Ly 3.2 antisera and complement (C)

Cells*	Pretreatment†	CMC‡ c.p.m.	CMC‡ %	CMC‡ % Reduction
Non-immune SPC	Diluent only	2,657	0	—
Immune SPC	Diluent only	288	89	—
Immune SPC	Normal rabbit serum+C	354	87	Standard
Immune SPC	Ra-*nu*/*nu*+C	2,447	8	91
Non-immune NAPC	Diluent only	8,565	0	—
Immune NAPC	Diluent only	1,072	87	—
Immune NAPC	Normal rabbit serum+C	1,178	86	Standard
Immune NAPC	Ra-*nu*/*nu*+C	6,992	18	80
Immune NAPC	Normal mouse serum+C	1,255	85	Standard
Immune NAPC	Anti Ly 3.2+C	7,043	18	80
(Immune NAPC Ra-*nu*/*nu*+C)+(Immune NAPC anti-Ly 3.2+C)		6,900	19	77

*B6 donors received a single subcutaneous injection of 2×10^7 BALB/c Meth A sarcoma cells (immune SPC) or multiple intraperitoneal injections of 5×10^6 BALB/c (immune NAPC) or B6 syngeneic (non-immune) spleen cells. SPC were collected 10 d after an injection and NAPC 3 d after the last injection of four consecutive weekly injections.

†For pretreatment, equal volumes of cells (10^7 ml^{-1}), antiserum (Ra-*nu*/*nu* diluted 1:2, anti-Ly 3.2 diluted 1:15) and complement (C; guinea pig serum at 1:3 with Ra-*nu*/*nu* antiserum and rabbit serum at 1:15 with Ly 3.2 antiserum) were incubated in 5% CO_2 in air for 45 min at 37 °C The cells were then washed twice and resuspended in culture medium with or without adjustments to the same concentration of viable cells: in all cases similar results were obtained. Earle's balanced salt solution with 2.5% foetal bovine serum and 5×10^{-5} M 2-mercaptoethanol was used for cell preparation and as diluent for antisera and C.

‡^{3}H-proline CMC microassay[21], in which the radioactivity of surviving attached monolayer target cells is measured. Washed SPC or NAPC (pretreated as described above; 10^5 per well) were added to a ^{3}H-proline labelled monolayer of BALB/c sarcoma cells (10^3 per well) for 12 h. CMC percentage and percentage reduction of CMC were calculated as described previously[10].

called the antigenic determinant(s) present on cytotoxic T cells, mouse killer T-cell antigen (MKTCA).

The antiserum was prepared by intravenous immunisation of a New Zealand rabbit with spleen cells of *nu*/*nu* mice, known to be deficient in thymus and T cells[6] but not in T-cell precursors[7,8]. Male *nu*/*nu* mice that served as donors of SPC were derived from a stock that has undergone 4–5 backcrosses to BALB/c mice. The inoculation of 1.7×10^8 *nu*/*nu* SPC was followed 7 d later by additional inoculation of $7 5\times10^8$ cells. The rabbit was bled 1 week after the second inoculation. The serum was heat inactivated for 30 min at 56 °C, diluted 1 : 4 with medium 199 and absorbed repeatedly *in vitro* for 30 min on ice with 10–20% (v/v) of packed cells. After one absorption with erythrocytes and one with thymic leukaemia EL4, six consecutive absorptions with thymocytes of different strains were carried out to remove activity against thymocytes as assessed in complement (C)-dependent cytotoxicity assay[9]. Absorbed antiserum, now called Ra-*nu*/*nu* was divided into small aliquots and kept at −70 °C until used.

Our earlier work[9] established that Ra-*nu*/*nu* antiserum possesses two different specificities. In addition to activity against bone marrow-derived lymphocytes (B cells) as demonstrated by inhibition of IgM plaque-forming cells and by cytotoxicity against myelomas, the antiserum has strong activity against cytotoxic T cells[9]. Activity against B cells can be absorbed with foetal liver cells but not with lymph node cells[9] and activity against cytotoxic T cells can be absorbed with lymph node cells but not with foetal liver cells and thymocytes (see below).

Results of typical experiments demonstrating strong inhibition of CMC by pretreatment with Ra-*nu*/*nu* antiserum and C of alloimmune spleen cells and non-adherent peritoneal lymphocytes are shown in Table 1. T-cell dependence of CMC is indicated by the strong suppression of the reaction by pretreatment with anti-Ly 3 serum (specificity anti-Ly 3.2), which is specific for alloimmune cytotoxic T cells[10,11]. We have shown previously[10] that inhibition of CMC in our system is due to the elimination of T cells with antibody and C, and not to the nonspecific blocking by antigen–antibody complexes which may be operative in systems measuring non-T cell killing[12]. Moreover, the elimination of B cells by pretreatment with various anti-B-cell specific sera and C, had no effect on CMC (H. S., unpublished). It might be argued, however, that cooperation of other cells is necessary for CMC and that inhibition of the reaction by Ra-*nu*/*nu* antiserum and C is due to elimination of the accessory non-T cells. The failure to reconstitute CMC by mixing cells pretreated with anti-Ly 3 serum and C with cells pretreated with Ra-*nu*/*nu* antiserum and C (compare line 11 with lines 8 and 10) rules out this possibility and indicates that cells sensitive to both antisera belong to the same subpopulation of T cells. The evidence that Ly 3 and MKTCA antigens are not the same comes from absorption experiments reported below.

In contrast to killer T cells, those carrying out the other functions tested remained unaffected by pretreatment with Ra-*nu*/*nu* antiserum and C. Table 2 shows results demonstrating the lack of inhibition of helper effect. This lack of inhibition was similarly observed in the case of the mitogenic response of spleen cells to PHA and con A and in the case of the G*v*H reaction of parental spleen cells injected into newborn F$_1$ hybrids (data not shown). Each function was tested in two or more independent experiments; in all cases similar results were obtained. These results indicate that killer cells differ in their surface antigenic properties from T cells cooperating with B cells in the antibody response, from cells responsible for G*v*H reactions and from cells responding to PHA and con A. This confirms and extends the evidence[1,11,13–16] that cells

Table 2 Helper effect: lack of inhibition by Ra-*nu*/*nu* antiserum and C

No. of primed cells per culture	Pretreatment of primed cells†	No. of TNP–PFC per culture on day 4* Expt *a*	Expt *b*
0	—	750	150
1×10^6	Diluent only	4,000	3,400
2×10^6		7,250	5,900
1×10^6	Normal rabbit serum +C	3,600	2,000
2×10^6		5,250	4,500
1×10^6	Ra-*nu*/*nu*+C	5,250	2,050
2×10^6		6,000	4,550

*Three B6 mice were injected intravenously with 0.1 ml 0.01% (v/v) sheep red blood cells (SRBC) After 4 d their spleens were pooled and pretreated with sera and C as described. Mishell–Dutton-type cultures were prepared containing increasing numbers of these pretreated primed cells (as indicated) with unprimed cells maintaining constant total 10^7 cells per culture. The cultures were sensitised with trinitrophenyl (TNP)-conjugated SRBC and assayed 4 d later for TNP-specific plaque-forming cells (TNP–PFC)[22]

†See Table 1.

causing CMC belong to a distinct subpopulation of functional T cells.

To determine the tissue distribution of MKTCA, absorption experiments were carried out. Results are summarised in Table 3. They show that MKTCA is present on cells from the lymph nodes and bone marrow of normal mice, as well as on spleen cells from *nu/nu* mice. It is absent, however, from normal foetal liver cells and thymocytes. This pattern of tissue distribution is different from that of Ly 3 alloantigen[17], indicating that although both antigens are characteristic of alloimmune killer cells, they are not the same. The relationship of MKTCA and Ly 3 antigens to Ka—another heteroantigen present on alloimmune killer cells[18]—is not known, as no other functional T cells have been examined for the presence of this antigen.

Table 3 Summary of absorption experiments with Ra-*nu/nu* antiserum showing the distribution of MKTCA antigen in lymphoid and haemopoietic tissues

Cells from	No. of cells used for absorption*	% Reduction in CMC†	Result of absorption
Thymus	5×10^8	72	–
Lymph nodes	5×10^8	9	+
Bone marrow	2.5×10^8	–2	+
nu/nu spleen	3.5×10^8	–16	+
Foetal liver	3.5×10^8	88	–

*Ra-*nu/nu* antiserum (0.5 ml) was absorbed with indicated number of cells for 30 min on ice. After absorption serum was diluted 1:2 and tested against non-adherent peritoneal cells (NAPC) from H-2-primed mice as described in Table 1.

†Mean value calculated from two to three experiments.

It is known that bone marrow from normal adult mice as well as spleen cells from *nu/nu* mice contain precursors of T cells[7,8,19]. Thus the ability of cells from these tissues to absorb anti-MKTCA activity suggest that the antigen is also present on the prethymic precursors of killer T cells.

Differentiation of T cells is generally depicted as involving transformation of prethymic cells to immunoincompetent thymocytes, which themselves are precursors of functionally mature, immunocompetent T cells[20]. The absence of MKTCA from thymocytes thus raises several possibilities which may have implications for the understanding of differentiation pathways of killer T cells. MKTCA should therefore prove to be a useful marker for further delineation and clarification of the differentiation stages involved in the process of generation of functionally distinct subpopulations of T cells. Although the majority of functional assays were carried out with cells of B6 mice, preliminary experiments in BALB/c mice and the fact that the antiserum has been produced in a heterologous system suggest that MKTCA is also characteristic of killer T cells of other strains.

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Virus-specific T-cell-mediated cytotoxicity across the H–2 barrier to virus-altered alloantigen

ALTHOUGH the biological function of the structures coded in the major murine histocompatibility (H–2) gene complex is poorly understood, some of these structures are crucially involved in most, if not all, cell-mediated immune functions[1,2]. To express their immunological function, thymus-derived lymphocytes (T cells) from conventional mice and the cells with which they interact must share part of the H–2 gene complex. T helper cells and bone marrow-derived lymphocytes (B cells) usually must be compatible for the *I* region of H–2 (ref. 3) to act cooperatively in T-cell dependent antibody responses. This region codes for Ia cell surface antigens and immune response (*Ir*) genes. Also, delayed type hypersensitivity to fowl γ-globulin can be transferred adoptively to intact mice only within *I*-region compatible donor-recipient combinations[4]. Distinct from these models, virus-immune T cells lyse virus-infected target cells *in vitro* only when both are compatible at the *K* and/or *D* regions of H–2 (refs 5–7). *I*-region compatibility is neither necessary nor sufficient on its own. Also T-cell-mediated lysis of chemically modified targets[8] and cytotoxicity against cells expressing either the minor histocompatibility antigens[9] or the male Y antigen[10] are similarly restricted.

Two probably mutually exclusive hypotheses have been proposed to explain this apparent restriction[5,6]. First, according to the 'physiological interaction' hypothesis, T cells specifically recognise antigens on the target cell surface; however, for cooperation or lysis to occur, self to self-like interactions are necessary. The structures responsible for such physiological interactions would have to be coded in the *I* region of H–2 for T helper cell functions[3], but in *K* and *D* for cytotoxic interactions[5,6]. Second, the altered self hypothesis proposes that T cells interact with other cells exclusively by way of the immunologically specific T-cell receptor. For cytolytic interactions this receptor is specific for virally, or for chemically or genetically (alloantigens), altered self structures coded in *K* and *D* regions (refs 5 and 6). On T helper cells this receptor may be specific for antigen-altered structures which are coded in the *I* region of H–2.

Most of the experimental evidence—at least that derived from competitive inhibition experiments *in vitro*[5] using the murine lymphocytic choriomeningitis virus (LCMV) model, selective proliferation studies *in vivo*[5] and experiments with H–2 mutant mice[1,12]—is explained best by the altered self idea. Also, cytotoxic activity against alloantigens (being a form of altered self) could be accommodated readily within this concept[5,6,12]. A physiological interaction hypothesis, however, is a possible explanation if the expression of physiological interaction structures is controlled by very complex genetic regulatory mechanisms[11,12].

If virus-specific cytotoxic T cells are specific for altered self,

Table 1 Virus-specific cytotoxic activity in spleens of LCMV-infected P→F_1 bone marrow chimaeras[*]

Spleen cells from LCMV-infected mice[†] (H–2 type)	Cell treatment (% dead cells after treatment)	% ^{51}Cr release from target cells[‡]					
		L929 ($H–2^k$)			J774 ($H–2^d$)		
		LCMV infected		Uninfected	LCMV infected		Uninfected
		10:1	3:1	10:1	10:1	3:1	10:1
C3H→C3D2 F_1 ($H–2^k$) ($H–2^k \times H–2^d$)	None (7)	67[‖]	51[‖]	24	98[‖]	73[‖]	41
	Anti-$H–2^k$+C′(> 98)	27	26	26	35	38	40
	Anti-$H–2^d$+C′(12)	68[‖]	50[‖]	26	85[‖]	66[‖]	40
	Normal B10.D2+C′ (13)	64[‖]	48[‖]	25	86[‖]	66[‖]	40
C3H ($H–2^k$)	None (5)	59[‖]	42[‖]	29	43	40	41
	Anti-$H–2^k$+C′(> 98)	25	24	23	—	ND	—
	Anti-$H–2^d$+C′ (10)	58[‖]	40[‖]	24	—	ND	—
	Normal B10.D2+C′ (12)	56[‖]	39[‖]	24	—	ND	—
C3H Normal		24	ND	24	40	ND	40
BALB/c ($H–2^d$)	None (12)	28	25	25	88[‖]	72[‖]	42
	Anti-$H–2^k$+C′ (15)	—	ND	—	85[‖]	63[‖]	41
	Anti-$H–2^d$+C′ (> 98)	—	ND	—	39	38	40
	Normal B10.BR+C′ (16)	—	ND	—	84[‖]	64[‖]	39
BALB/c Normal	None	27	ND	25	38	ND	30

[*] Mice were obtained from Jackson Laboratories, Bar Harbor, Maine and used at 7–10 weeks of age. (C3H×DBA/2) F_1 were irradiated with 900 r and reconstituted with 2×10^7 anti-θ+C′-treated C3H bone marrow cells Chimaeras were infected intravenously with 10^3 intracerebral LD_{50} of the WE strain of LCMV 11 weeks later. 7-d immune spleen cells were used.

[†] Anti-H–2 sera were obtained by hyperimmunising B10 BR ($H–2^k$) mice with B10.D2 ($H–2^d$) spleen cells and vice versa. Both antisera killed > 90% specifically when assayed at a 1:30 dilution and 5×10^6 spleen cells ml^{-1}. A selected rabbit complement was used unabsorbed at a 1:8 final concentration. After the treatment viability was determined by Trypan blue exclusion. The antisera were used at a 1:3 final dilution and 5×10^6 spleen cells ml^{-1}. The anti-H–2 sera were highly specific as indicated by the percentage of dead cells found after treatment. Cells were resuspended according to the original viable cell concentration before treatment (groups none) and assayed. Decrease of activity by nonspecific antiserum+C′ was not different from that caused by normal control serum+C′.

[‡] ^{51}Cr release assay was carried out as described previously[5,6]; J774 target cells were provided by Dr M. Bevan, Salk Institute, La Jolla, California. Spleen cells were assayed at the spleen cell–target ratios of 10:1 and 3:1 for 12 h at 37 °C; results are means of triplicates; s.e.m. was 0.4–1.5 for L929 cells and 0.7–2.4 for J774 cells. LCMV-immune spleen cells tested on infected targets caused statistically significant ^{51}Cr release when compared with release from normal targets or with the activity of normal spleen cells.

[‖] $P < 0.01$.

ND, Not determined.

it should be possible to sensitise T cells specifically to virus-altered alloantigen across the H–2 barrier. This report demonstrates that virus-specific cytotoxic T cells from chimaeric mice can lyse across the H–2 barrier only those infected allogeneic targets to which they are tolerant. Thus, reactivities to virus-specific altered self, to virus-altered alloantigen or to alloantigen itself are obviously comparable[5,6,12].

To overcome the strong normal reactivity to alloantigens that could mask potential but experimentally undetectable reactivity for virus-altered alloantigen, alloantigen tolerant P→F_1 hybrid irradiation chimaeras reconstituted with bone marrow of one parent were used[13]. LCMV-infected C3H→(C3H×DBA/2) F_1 chimaeras generated cytotoxic T cells, with exclusively $H–2^k$ surface characteristics, which lysed

Table 2 T-cell dependence of cytotoxic activity of LCMV-infected P→F_1 bone marrow chimaeras[*]

Spleen cells from LCMV-infected mice[*] (H–2 type)	Cell treatment[*] (% dead cells after treatment)	% ^{51}Cr release from LCMV-infected target cells[†]		
		L929 ($H–2^k$)	J774 ($H–d^d$)	C57BL/6[‡] ($H–2^b$)
C3H→C3D2 F_1 ($H–2^k$) ($H–2^k \times H–2^d$)	None (15)	49[‖]	60[‖]	35[¶]
	Anti-$H–2^k$+C′ (> 98)	14[¶]	24[¶]	ND
	Anti-$H–2^d$+C′ (18)	48[‖]	57[‖]	ND
	Normal AKR+C′ (19)	46[‖]	58[‖]	ND
	AKR anti-θ C3H+C′ (54)	15[‖]	25[¶]	ND
C3D2 F_1 ($H–2^k \times H–2^d$)	None (14)	54[‖]	61[‖]	36[¶]
	Anti-$H–2^k$+C′ (> 97)	16[¶]	24[¶]	ND
	Anti-$H–2^d$+C′ (> 95)	14[¶]	25[¶]	ND
	Normal AKR+C′ (18)	52[‖]	60[‖]	ND
	AKR anti-θ C3H+C′ (50)	17[¶]	23[¶]	ND
C57BL/6 ($H–2^b$)	None (16)	16	26	87[‖]
Normal C3H → C3D2 F_1	—	15	23	37
Normal C3D2 F_1	—	15	21	38
Normal C57BL/6	—	17	27	35

[*] Mice, immunisation and antisera, see Table 1. AKR anti-θ C3H was purchased from Bionetics, Kensington, Maryland 20795 (Cat 8301-01, Lot No 231-61-5) With a selected rabbit complement C′ thymocytes were lysed to 50% at a 1:40 dilution of the anti-θ antiserum. The antiserum was used at a 1:8 final dilution In these conditions anti-SRBC PFC were not lysed[5].

[†] The cytotoxicity assay was carried out as described previously[5,6]. Spleen cells were assayed at a 30:1 ratio for 6 h at 37 °C. Results are means of triplicates; s.e m were 0.3–0.9 for L929, 0.5–1.7 for J774 and 0.9–2.2 for C57BL/6 macrophage targets. LCMV-immune spleen cells caused statistically significant lysis of H–2 compatible or the tolerant H–2 haplotype targets; treatment with irrelevant anti-H–2 antiserum+C′ or control sera + C′ did not cause loss of activity Treatment with AKR anti-θ C3H+C′ or with the relevant anti-H–2 antiserum abolished all activity; residual activity was not significantly different from normal spleen cells on infected targets.

[‡] Peritoneal macrophages from normal mice were used as targets as described previously[5].

[‖] $P < 0.01$.

[¶] Not significant.

ND, Not determined.

virus-infected H–2^d target cells but not uninfected H–2^d targets (Table 1). These T cells are tolerant for H–2^d, but can readily react to virus-altered alloantigen. Comparable results were obtained in separate experiments with five C3H→(C3H×DBA/2)F$_1$, some infected with vaccinia virus instead of LCMV (R.M.Z., unpublished). The cytotoxic effector cells of H–2^k type from chimaeric mice are T cells, as shown by their susceptibility to AKR anti-θ C3H+complement treatment (Table 2). This notion is further supported by the fact that they lysed infected target cells of the tolerant allogeneic parental H–2^d type but were still H–2 restricted in that they could not lyse infected targets of an unrelated allogeneic H–2^b haplotype (Table 2). These results add strong circumstantial evidence in favour of the altered self hypothesis. Our data suggest that altered self, alloantigen, and altered alloantigen are comparable antigens, since all are attacked by T cells because they differ from the normal unaltered cell-surface self marker.

The interaction model has been extended to accommodate similar findings in which T helper cells from chimaeric mice collaborated with H–2*I* region-incompatible B cells[13,14]. In this case, structures of T cells that interact with H–2-incompatible cells may be allowed to differentiate according to the H–2 environment in which they circulate in tolerant conditions[15]. To accommodate the crossing of the H–2 barrier by LCMV or vaccinia immune T cells of chimaeras, interaction structures on these T cells for the tolerant H–2 haplotype could, theoretically, have undergone similar adaptive differentiation. Such a mechanism, however, in addition to the complex genetic controls previously mentioned[11,12] seems to be very complicated. The concept that cytotoxic T cells are specific for altered cell-surface markers coded in *K* or *D* of H–2, and that T helper cells may be specific for antigen-altered cell surface structures coded in the *I* region, seems to be the simplest and most generally applicable explanation. Nevertheless, the physiological interaction idea cannot be disregarded until either altered *K*- or *D*-coded structures or physiological interaction structures are defined biochemically.

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Cytotoxic T lymphocytes recognise allogeneic tolerated TNP-conjugated cells

DURING a cytotoxic response, T cells can recognise and react to cell-associated virus antigens[1], minor histocompatibility antigens[2,3] or trinitrophenyl(TNP)-conjugated cell-surface components[4] only when responder, stimulator and target cells share either K- or D-region determinants of the major histocompatibility complex (MHC). Two possible explanations for these observations have been discussed: first, cytotoxic T cells recognise non-H–2 antigens but to lyse targets they have to interact with self antigens controlled by the MHC on stimulator and target cells (cell-interaction determinants), and second, T-cell clones recognise and react to new antigenic determinants created by the association of MHC gene products with non-H–2 antigens. We present evidence here that T cells which have matured in a semi-allogeneic environment can recognise, in a specific way, TNP in association with tolerated allogeneic cells not sharing *H–2K* or *H–2D* loci with the responding cells.

DBA/2 T cells, tolerant to CBA/J alloantigens were obtained from chimaeric mice, produced by injecting anti-θ-treated DBA/2 bone marrow cells into 900-R irradiated F$_1$ (CBA/J×DBA/2) hybrids (DBA → CBA F$_1$– chimaeras)[5]. Five months after irradiation, chimaeric mice as well as (CBA/J×DBA/2) F$_1$ hybrids were immunised with TNP-conjugated CBA spleen cells (TNP–CBA). TNP was conjugated to either spleen cells or ^{51}Cr-labelled LPS blasts according to a slight modification of the method of Shearer *et al*[4], using a cacodylate buffer, *p*H 7.3, containing 10 mM 2,4,5-trinitrobenzene sulphonate (1 ml per 10^8 viable nucleated cells).

The *in vitro* assay of cell-mediated lympholysis (CML)[6] as well as the assay for testing the lymphoid cell chimaerism of bone marrow chimaeras[7] have been described before.

Using H–2^k anti-H–2^d and H–2^d anti-H–2^k sera the lymphoid cell chimaerism of DBA → CBA F$_1$ chimaeras was tested. As Table 1 shows, all cells from lymph nodes were killed by anti-H–2^d sera whereas no killing above background was observed with anti-H–2^k sera, indicating the absence of F$_1$ hybrid host cells.

Table 1 Killing of cells

	Incubation with		
Lymph node cells	Anti H–2^k + C′	Anti H–2^d + C′	Saline + C′
DBA/2	5%	97%	8%
(CBA×DBA/2) F$_1$	83%	96%	9%
Chimaera CBA → DBA F$_1$	8%	97%	9%

To test the reactivity of cells from DBA → CBA F$_1$ chimaeras as well as from (CBA/J×DBA/2) F$_1$ hybrids in CML, spleen cells were prepared and restimulated with either TNP–CBA stimulators or stimulated with SJL (H–2^s) cells *in vitro*. After 5 d, cultures were tested for cytotoxicity directed against TNP–CBA, CBA, TNP–DBA/2 or SJL ^{51}Cr-labelled target cells. (CBA/J×DBA/2) F$_1$ cells restimulated *in vitro* with TNP–CBA lysed TNP–CBA targets (Fig. 1*a*) but neither CBA, TNP–DBA/2 nor SJL targets (Fig. 1*b–d*). The same responder cells stimulated *in vitro* with SJL cells lysed SJL targets but not CBA targets and to some extent also TNP–CBA and TNP–DBA/2 targets. This apparently nonspecific lysis of TNP-conjugated targets by cell suspensions containing high levels of cytotoxic activity has been found consistently, regardless of the strain combinations used. This is probably caused by nonspecific adherence of cytotoxic T cells to TNP-coupled targets. The assumption is supported by the fact that TNP–CBA-induced cytotoxic lymphocytes do not lyse non-conjugated target cells as SJL target cells (Fig. 1*d*). A result basically similar to that with TNP–CBA-primed F$_1$ responder cells was obtained with spleen cells from a DBA→CBA F$_1$ chimaera. Restimulation with TNP–CBA *in vitro* induced cytotoxic activity directed against TNP–CBA (Fig. 2*a*) but neither CBA, TNP–DBA/2 nor SJL targets (Fig. 2*b–d*). Stimula-

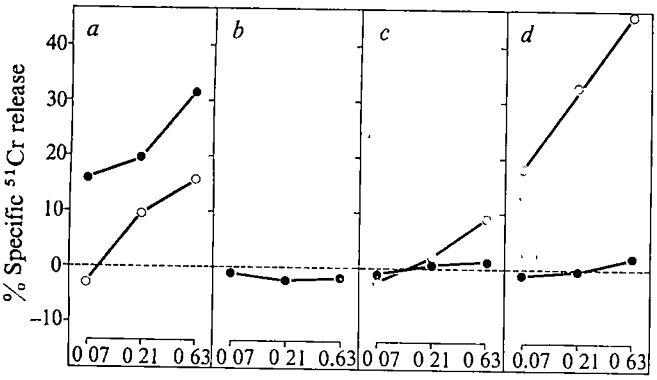

Fig. 1 Killing of TNP–CBA (*a*), CBA (*b*), TNP-DBA/2 (*c*) and SJL (*d*) targets by (CBA × DBA) F_1 spleen cells immunised *in vivo* with TNP–CBA spleen cells and restimulated *in vitro* with either TNP–CBA (●) or SJL (○) stimulating cells. The abscissa shows the known number (×10^6) of responders cultured on day 0, the descendants of which are the unknown number of killer cells added to each well containing 2 × 10^4 target cells. Each point represents the arithmetic mean of triplicate cultures. The standard deviation is less than 3%. The spontaneous ^{51}Cr release of the various targets varied between 20 and 31%.

tion with SJL cells resulted in lytic activity directed against SJL targets only (Fig. 2). On a per cell basis cytotoxicity of chimaeric cells compared with F_1 hybrid cells was lower on all targets. This is probably why nonspecific killing of TNP-coupled target cells was not seen.

Our experiments confirm conclusions drawn from work done using different experimental conditions: Doherty and Zinkernagel[8,9] as well as Shearer *et al.*[4] showed that F_1 hybrid cells sensitised to virus-infected or TNP-conjugated cells of one parental strain would only lyse 'modified' cells of that parental strain but not of the other. In addition it was shown that either H-2D or H-2K homology between modified stimulator and target cells was sufficient for generation of cytotoxicity. According to the hypothesis postulating the essential role of some MHC homology between effector and target cells for killing of modified targets one would have to postulate two separate 'cell interaction loci' which would have to be under allelic exclusion This unlikely hypothesis can be rejected on the basis of the experiments reported here, which indicate that homology of MHC products between effector and target cells is not required to obtain killing of TNP-conjugated target cells, whereas homology of MHC products between TNP-conjugated stimulator and target cells is essential. Our experiments thus show that precursors for cytotoxic T cells are triggered and react to antigenic determinants created by association of MHC gene products with non-H-2 antigens (often referred to as 'altered H-2 antigens'). In what way these determinants are formed and recognised by T cells is unknown, and the subject of speculation[10,11].

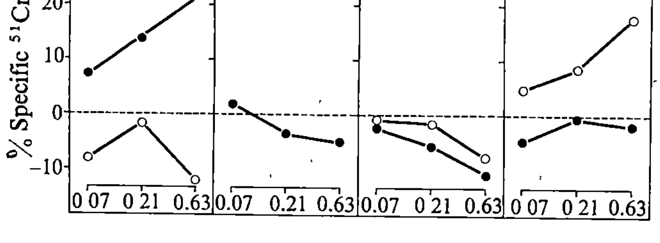

Fig. 2 Killing of TNP–CBA (*a*), CBA (*b*), TNP–DBA/2 (*c*) and SJL (*d*) targets by spleen cells from a DBA→ CBA F_1 chimaera immunised *in vivo* with TNP–CBA cells and restimulated *in vitro* with either TNP–CBA (●) or SJL (○) stimulating cells. Figures on the abscissa indicate the number (× 10^6) of responders per well (see Fig. 1).

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Binding of human immunoglobulins to pituitary ACTH cells

WE report the unexpected finding that the adrenocorticotropic hormone (ACTH)-secreting cells of the human anterior pituitary gland show an affinity for human immunoglobulins resembling that of normal mast cells for IgE. This phenomenon could have physiological significance if considered in relation to the messages leading to synthesis or release of the hormone.

In the course of studies which led to the identification of antibodies directed against the prolactin-secreting cells of the adenohypophysis in patients suffering from autoimmune polyendocrine deficiencies[1], it was found that most human sera exhibited a cytoplasmic immunofluorescence (IFL) on ACTH-secreting cells. The type of endocrine cell was identified by a double IFL sandwich technique in which the patients' sera were followed by an anti-immunoglobulin conjugated with fluorescein isothiocyanate (FITC)(green) and a rabbit antibody specific for ACTH counterstained with a rhodamine-labelled conjugate (red) on the same section. None of the other five types of anterior pituitary cells reacted in this way, and anti-human Ig conjugates applied by themselves did not produce any fluorescence on ACTH cells. The three main immunoglobulin classes in human serum were reactive, although the strongest staining was usually seen with anti-IgM conjugates.

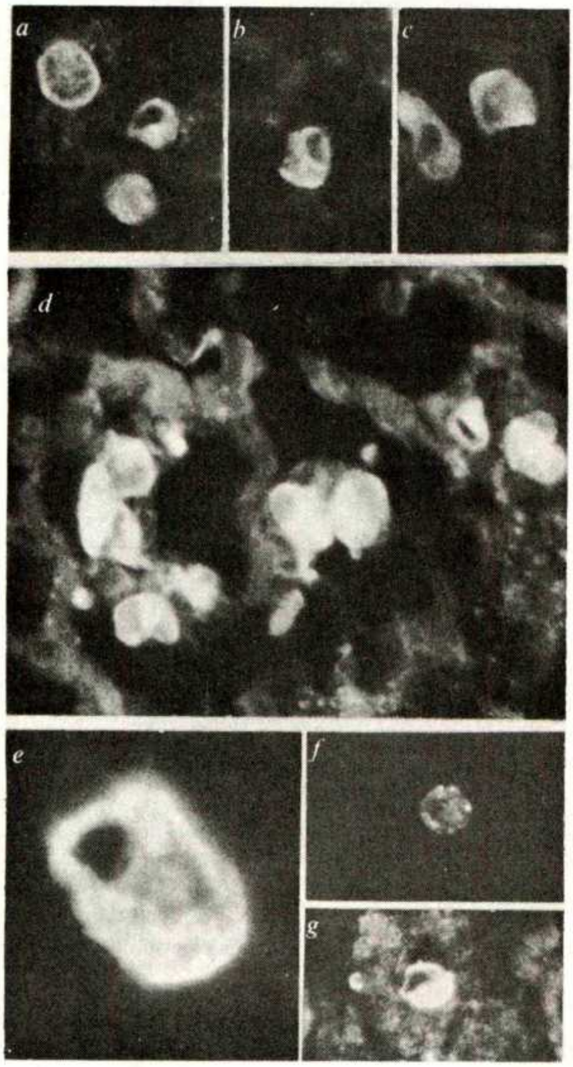
Experiments carried out to investigate this phenomenon suggested that the binding occurred through the Fc portion of the immunoglobulins and was not an antigen–antibody reaction. Table 1 shows the Ig preparations applied in these experiments. Four separate pools of mixed human Ig stained the ACTH cells. Two preparations of Fc fragments[2] from separate Ig pools were fully reactive, whereas the Fab from the same pools gave negative results using appropriate conjugates. One Fc and one Fab preparation made from normal pooled IgG failed to stain ACTH cells, indicating that the binding of IgG is of relatively low affinity and requires aggregates which cannot form with Fc fragments. A similar situation exists in relation to Fc receptors on lymphocytes. The strongest IFL was obtained with IgM in all categories of sera tested. Weaker staining was generally seen with IgG and IgA. One serum containing 30,000 IU IgE ml^{-1} and several sera with normal IgE content gave negative results when tested with an anti-IgE conjugate. One IgD myeloma serum similarly failed to react when tested with anti-IgD. The staining of ACTH cells was positive when fresh human sera were followed by an anti-β_{1c} conjugate, showing that the binding Fc region was com-

Table 1 Analysis of Ig binding to pituitary ACTH cells

No. of preparations used	Type of Ig preparation	Antiserum (conjugated with FITC)	IFL on ACTH cells
4	Pools purified H-Ig (Cohn fraction II)	Sheep anti-H-F(ab)$_2$ (laboratory preparation)	+
	1 Laboratory preparation		
	3 Lister Institute preparation	Goat anti-H-Fc (Nordic)	+
2	F(ab)$_2$ fragments from pooled H-Ig	Sheep anti-H-F(ab)$_2$ (laboratory preparation)	−
1	F(ab)$_2$ fragments from pooled H-Ig	Sheep anti H-F(ab)$_2$ (laboratory preparation)	−
2	Fc fragments from pooled human Ig	Goat anti-H-Fc (Nordic)	+
		Sheep anti-H-Fc (Wellcome)	+
1	Fc fragments from pooled H-IgG	Goat anti-H-Fcγ (Nordic)	−
1	Purified μ chain (μ-chain disease) (Dr E. C. Franklin)	Goat anti-Fc μ (Nordic)	−
1	Whole serum (μ-chain disease)	Sheep anti-Fc μ (Wellcome)	−
1	Monoclonal IgM-κ, 19S fraction	Goat anti-Fc μ (Nordic)	+
1	19S fraction of NHS	Goat anti-Fc μ (Nordic)	+

plement fixing. Most sera showed titres between 1 and 80. Sera from cases of Waldenstrom's macroglobulinaemia were applied to determine whether the staining was dependent on any special features of the IgM tested. One of these sera gave a titre greater than 10,000 and the purified 19S fraction was equally reactive. This high reactivity, together with the fact that all anti-human Ig conjugates were effective —although they were raised in different animal species injected with separate pools of Ig—excludes the possibility that this IFL is due to a serum protein other than an immunoglobulin to which the animals might have developed adventitious antibodies. Further evidence is provided by the positive β_{1c} reaction and the negative tests obtained with most hypogammaglobulinaemic sera.

Fig. 1 Human pituitary stained by immunofluorescence with normal human serum (Fc binding). *a–d*, Pituitary from Cushing's disease. Crooke cells show variable cytoplasmic staining. *e*, Single Crooke cell at high magnification. *f*, Live ACTH cell showing interrupted membrane fluorescence. *g*, Normal post mortem pituitary with small ACTH cell.



All human pituitaries showed this ACTH cell fluorescence, including six post mortem glands, and seven hypophysectomy specimens, five from carcinoma of breast cases and two removed for Cushing's disease. The reaction was still positive after fixation of the pituitary with acetone or methanol. Animal pituitaries are being tested but no conclusive results can as yet be given.

On unfixed cryostat sections of normal pituitary the ACTH cells were small with eccentric nuclei, and showed a uniform smooth staining pattern involving the entire cytoplasm (Fig. 1*g*). In Cushing glands containing numerous Crooke cells, the staining was intense and of variable pattern (Fig. 1*a–e*). When a suspension of mixed live pituitary cells was examined, normal sera gave an interrupted membrane fluorescence on cells which, after fixation, could be shown to react with anti-ACTH by the double IFL technique (Fig. 1*f*).

Results obtained with 430 individual human sera are shown in Table 2. All were first tested with anti-IgM and negative sera were retested with anti-IgG and anti-IgA; only those failing to stain ACTH cells with all three conjugates were listed as negative.

As might be expected, seven of the eight hypogammaglobulinaemic sera (supplied by Professor J. Soothill and Dr A. D. B. Webster) were completely negative. A weakly positive reaction was seen with one serum containing 13.9 IU IgM ml^{-1}. Seventeen normal sera from laboratory staff all stained ACTH cells but five healthy relatives belonging to a family with two cases of idiopathic hypoparathyroidism were surprisingly negative; and it is notable that 6 out of 15 hypoparathyroid sera also failed to stain these

Table 2 Binding to ACTH cells of human pituitary by the immunoglobulins in pathological and normal sera

Diagnosis	No. of sera tested	Negative fluorescence of ACTH cells
Hypopituitarism	20	3
Addison's disease	112	14
Hypoparathyroidism	15	6
Rheumatoid arthritis	80	11
Hypogammaglobulinaemia	8	7
Waldenstrom's macroglobulinaemia	31	2
Other conditions	143	4
Normal subjects	22	5*
Total	430	52 (12%)

* Normal relatives of hypoparathyroid cases.

cells, although they were not hypogammaglobulinaemic. Among the other categories of patients tested, the percentage of negative reactors varied from 3 to 14%.

From these data we conclude that certain sites within the Fc region of immunoglobulin molecules have a non-immunological affinity for an unknown constituent present both on the cell membrane and in the cytoplasm of ACTH cells in the human anterior pituitary. This was an unexpected finding and its physiological significance is not yet understood.

The existence of Fc receptors on subpopulations of blood lymphocytes[3] and on mast cells and basophils, is established[4]. The fixation of IgE and of anaphylactic IgG on the mast cells by their Fc portions is necessary before the 'allergen challenge' stage of histamine release. This consists of attachment of the appropriate antigen and bridging of the free Fab portions of the Ig molecules; this process triggers the cells to release their granules. In the same way macrophages can be activated when immune complexes become attached to Fc receptors. Possibly a similar role might be found in future for the Fc receptors on ACTH cells. Note that Stanworth[5] has shown that a sequence of basic amino acid residues within the ACTH molecules is capable of inducing histamine release from normal mast cells in the absence of immunoglobulins.

For the present it is important to be aware of ACTH-cell IFL when looking for autoantibodies to anterior pituitary gland in human patients. Furthermore this reaction may be used to identify ACTH cells for histological purposes, because the Ig binding seems to be as specific for the identification of these cells as for anti-ACTH sera raised in animals. It will also be of interest to determine whether lung cancers and other tumour cells with inappropriate ACTH secretion show this affinity for immunoglobulins.

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Infection of a cell line of mouse L fibroblasts with scrapie agent

THE infection of cell cultures with the causative agents of subacute spongiform encephalopathies — scrapie, kuru, Creutzfeldt–Jakob disease and transmissible mink encephalopathy—has not yet been reported. The development of a cell line (SMB), from the brain of a mouse affected with scrapie, in which the agent multiplies has shown that replication *in vitro* is possible[1]. Studies with this cell line have, however, been limited because no control culture was available for comparative studies. This limitation has now, in part, been overcome by the successful infection of cultures of mouse L cells with scrapie agent, as described here.

The scrapie agent is closely associated with cellular fragments and attempts to release and purify the agent have been unsuccessful. Recently Millson *et al.*[2] have shown that approximately 98% of the membrane proteins from scrapie membrane preparations can be solubilised by treatment with lysolecithin, giving an increase of up to 100-fold in the concentration of the agent. It seemed worthwhile to attempt to infect cells with this partially 'purified' preparation because, first, the removal of a large proportion of the membrane components made it possible to introduce a higher concentration of agent into the cell cultures than previously, and second, as lysolecithin can cause cell fusion[3], it seemed possible that the association of bound lysolecithin with the particulate membrane fragments containing the scrapie agent might induce uptake of these fragments by the cells.

A membrane fraction was prepared from the brains of scrapie-affected mice by homogenising the tissue in 0.32 M sucrose to give a 10% suspension. Nuclei and unbroken cells were pelleted by centrifugation at 500*g* for 10 min. The post-nuclear supernatant fluid was centrifuged at 100,000*g* for 1 h and the sedimented membrane fraction was resuspended and homogenised in 25 ml of 0.05 M bicarbonate buffer, *p*H 8.3, containing 192 mg of lysolecithin. After incubation at 37 °C for 2 h, the sample was centrifuged at 100,000*g* for 1 h. The final pellet containing particulate membrane fragments was resuspended in 7 ml of Hanks' buffered salt solution. Dilutions of the suspension between 1/1 and 1/100 were then used to resuspend pellets of mouse L cells (derived from clone 929, Flow Laboratories) and the mixture was added to cell culture medium in medical flat bottles. The medium used was Eagle's minimum essential medium with 10% foetal calf serum.

The lysolecithin-prepared suspensions of membrane fragments were toxic to the L cells but some survived and two cultures were established. Culture *A* was from cells exposed to a suspension diluted 1/3 and culture *B* was from dilution 1/100. Monolayers were eventually obtained in each case about 6 weeks later. The monolayers were then submitted to 15 serial passages during a period of up to 29 weeks and on each occasion dilutions of the cells were made equal to $10^{-0.5}$.

The presence of scrapie activity at the 1st, 7th, 11th and 15th passages was determined by titration of samples of the cultures in mice as described before[4] (Table 1). In culture *A* there was a decline in titre from the 1st (3.3 $\log_{10}$ LD_{50} per 0.05 ml) to the 7th (1.6) passage and thereafter the titres remained at about the latter level. In culture *B* the titres varied between 1.8 and 1.1 from the first to the 15th passage. These titres correspond to about 3,000 cells per LD_{50}, which is a much lower level of infectivity than for the SMB cell line (100 cells per LD_{50}). The difference between the titre of the first passage of cultures *A* (3.3) and *B* (1.8) is the same as the difference in the dilutions of suspension used for the exposure of cells to scrapie infectivity. This suggests that at least some of the activity of the first passage was derived from the suspension of membrane fragments and not from multiplication of agent in the culture.

Table 1 Titre of scrapie agent in two cultures of mouse L fibroblasts at various passages after exposure

Culture	Passage			
	1	7	11	15
A	3.3*	1.6	1.0	1.8
B	1.8	1.6	1.5	1.1

* Titre of scrapie agent $\log_{10}LD_{50}$ per 0.05 ml.

The levels of scrapie activity in the original mixtures of membrane suspension and L cells were estimated at about $10^{5.5}$ (A) and $10^{4.0}$ LD_{50} per 0.05 ml (B) so that by the 12th and 9th passages, respectively, the original infectivity, if it had all been retained in the cultures at the first passage, would have been eliminated by the subsequent dilution of the cells at each passage. The presence of activity at later passages must, therefore, be due to multiplication of agent. Further, since each passage involved a dilution of cells, the observation of a similar level of activity at each passage titrated also indicated that the agent had multiplied.

Fusion of plasma membrane can occur as a consequence of exposure of cells to lysolecithin, and multinucleated forms may be seen[3]. During the development of the initial monolayers of L cells exposed to scrapie agent an increased number of multinucleated cells was noted, suggesting that fusion had occurred. The establishment of cultures in which the scrapie agent was replicating could have been due to the presence of lysolecithin which, bound to membrane fragments containing scrapie infectivity, fused with the L cells Fusion could also have resulted from the presence of free lysolecithin in the membrane suspension used to infect the cultures. Scrapie infectivity is associated with plasma membrane of SMB cells[5]; the successful infection of cell cultures in the presence of a substance known to cause fusion of membranes is consistent with the observation that the agent is located on the membrane.

Although the titres obtained in the cultures of L cells were low, the evidence that infection of cell cultures with scrapie agent has occurred is encouraging It suggests that further attempts to fuse membrane fragments from scrapie-affected tissue to cells should be made.

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Renal tubular adaptation to dietary phosphorus

THE kidney is very important in the maintenance of the overall balance of inorganic phosphate (P_i). The renal response to variations in dietary P_i has been generally explained by changes in the amount of P_i filtered by the glomerulus. When a change in the tubular P_i transport has been suspected, it has usually been attributed to an alteration in parathyroid hormone (PTH) secretion[1,2]. We have shown that the renal tubule changes its capacity to reabsorb P_i according to homoeostatic requirements independently of the filtered load of P_i (ref. 3). Since such a tubular adaptation also occurs in the absence of PTH (ref. 3), the question of the relative potency of PTH and that of dietary P_i in changing the tubular transport of P_i is of interest. Here we report that variations in dietary P_i cause a much greater change in the tubular capacity to transport P_i than removal of the thryoparathyroid glands.

The renal handling of P_i was studied in pairs of sham-operated (SHAM) and thyroparathyroidectomised (TPTX) rats fed diets containing either low (0.2 g % dry weight) or high (1.8 g % dry weight) amounts of phosphorus for ten days (see Table 1 legend). The fractional excretion of P_i ($C_{P_i}/C_{In} \times 100$), measured in steady-state conditions and at the same plasma levels of P_i ($[P_i]_{Pl}$), was about five times higher in SHAM than in TPTX rats on a high P_i diet (Table 1). As expected, calcaemia ($[Ca]_{Pl}$), measured during the clearance periods, was higher in SHAM than in TPTX animals (2.43±0.04 and 1.78±0.16 mM respectively, $P < 0.01$). We found no significant differences in the weight of the animals (182±3 and 174±5 g), in urinary volume (37±5 and 30±3 µl min^{-1}), or in C_{In} (1.29±0.06 and 1.33±0.04 ml min^{-1}). Similar results were obtained in SHAM and TPTX rats, on a low P_i diet (Table 1). The ratio C_{P_i}/C_{In} was twice as high in SHAM as in TPTX animals. $[Ca]_{Pl}$ was higher in SHAM than in TPTX rats (2.46±0.05 and 2.00±0.09 mM, $P < 0.001$). Again there were no significant differences in weight (170±5 and 169±4 g), in urinary volume (38±6 and 40±6 µl min^{-1}), or in C_{In} (1.33±0.07 and 1.29±0.10 ml min^{-1}). In addition, none of these latter parameters differed significantly from those of the two groups on high P_i intake.

Table 1 Variation in the fractional excretion of P_i in response to dietary P_i compared with thyroparathyroidectomy

Diet	$C_{P_i}/C_{In} \times 100$ SHAM	 TPTX	 SHAM–TPTX
High P_i (1.8 g % dry weight)	34.7±4.4 (15)	7 1±1.8 (7)	4.9
Low P_i (0.2 g % dry weight)	0.098±0.003 (7)	0.045±0 009 (7)	2.2
High/low P_i	354	157	

Numbers in parentheses correspond to number of animals. Fractional excretion of phosphate ($C_{P_i}/C_{In} \times 100$±s e.m.) was measured at equal plasma phosphate concentrations in pairs of rats fed high P_i (1.8 g % dry weight) and low P_i (0.2 g % dry weight) diets for 10 d. The mean (±s.e m.) intake of phosphorus was 31±1 and 276±13 mg d^{-1} for rats fed on a low and high P_i diet respectively. Dietary Ca^{2+} and Na^+ contents were kept constant (1.2 and 2.45 g % dry weight respectively). Surgical thyroparathyroidectomy (TPTX) or a sham operation (SHAM) was performed on the eighth day. The effectiveness of parathyroidectomy was evaluated after the clearance experiment; the rats were returned to the lab chow for 5 d, fasted overnight and their calcaemia ($[Ca]_{Pl}$) determined the following morning by atomic absorption spectrometry Data are only presented for TPTX rats which displayed a $[Ca]_{Pl}$ below 1.88 mM (= 7.5 mg %) in these conditions and which were therefore considered to be fully parathyroidectomised. On the day of the clearance measurement animals were infused intravenously (4 ml h^{-1}) with either isotonic NaCl or neutral P_i (16% NaH_2PO_4 and 84% Na_2HPO_4) at doses of 60 or 120 µmol h^{-1}. The osmolality of the solutions was adjusted to 300–310 mosmol l^{-1} by addition of NaCl where necessary. The clearances were measured in non-fasted conscious animals according to a technique described earlier[4]. Inulin was determined by the anthrone technique[5] and P_i by the method of Chen *et al.*[6]. Data were analysed only from values of $[P_i]_{Pl}$ in the range between 2.25 and 2.90 mM. The mean $[P_i]_{Pl}$ values of the four experimental groups were not significantly different: high P_i diet, SHAM 2.54±0 01 and TPTX 2.60±0.07 mM; low P_i diet, SHAM 2 46±0.09 and TPTX 2.49±0.11 mM.

Thus, independently of the filtered load, and according to the values of the SHAM–TPTX ratio in Table 1, the presence of PTH may increase the fractional excretion of P_i about 2–5 times. The greater effect due to the removal of the parathyroid glands under high P_i diet can be explained by parathyroid hyperfunction, occurring in this condition[2]. The fractional excretion of P_i can, however, be changed more than a hundredfold in both intact and TPTX rats by varying the previous dietary intake of P_i as expressed by the ratios of fractional clearances on the high and low P_i diets (Table 1).

The dietary P_i level, therefore, seems to control a mechanism which is independent of circulating PTH and which seems to be much more potent than this hormone in changing the renal tubular transport capacity of P_i. The present data indicate that PTH would amplify the important dietary-induced change in the tubular capacity for this anion by enabling more P_i to be excreted under a high P_i diet.

Differences induced by dietary input of P_i can be observed in the presence of the same calcaemia (see above) and the same urinary *p*H (ref. 3). It is maintained under marked expansion of the extracellular volume[3]. Renal adaptation to P_i is probably independent of vitamin D_3 and its metabolites since in TPTX vitamin D-deficient rats maintained on a low P_i diet[7], tubular reabsorption of P_i is complete.

Tubular adaptation to dietary P_i seems not to be mediated by the main factors generally believed to be most important in the regulation of the renal handling of P_i. Our results have revealed a homoeostatic mechanism of great potency which must contribute to the maintenance of P_i balance by the kidney.

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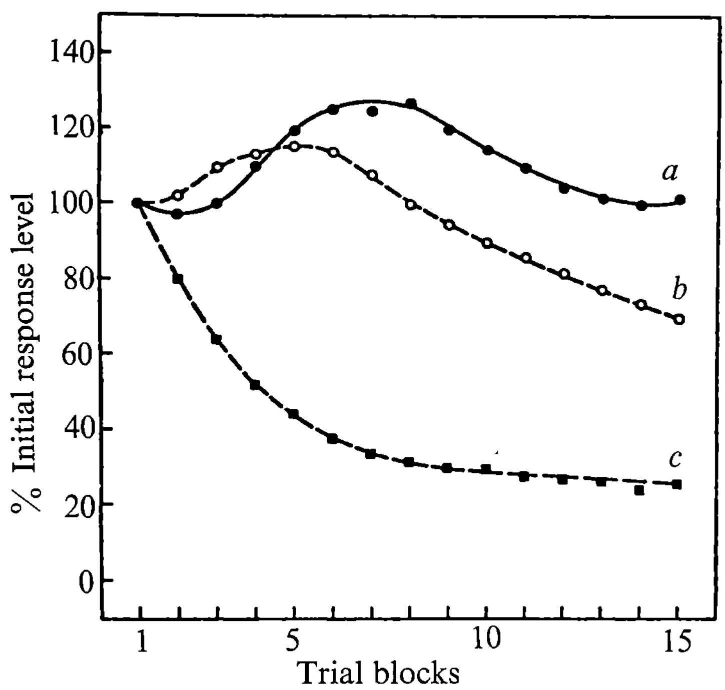
Computer simulation of a model of habituation

HABITUATION—in general defined as a reversible decrement of response to repeated stimulation—is a widely studied form of behavioural plasticity. The reversibility sets habituation apart from fatigue and accommodation. An habituated stimulus may once again be able to evoke a response after the presentation of an intervening novel stimulus. This phenomenon, known as dishabituation, rules out sensory and motor fatigue as the bases of the response decrement.

Thompson and Spencer[1] have described several characteristics which together form a widely accepted operational definition of habituation. Two of these are that relative response decrement, expressed as a percentage of the unhabituated response, proceeds more rapidly for less intense stimuli, and that both habituation and dishabituation recover spontaneously over time if the stimulus is withheld.

Although habituation has been studied at the neurophysiological level in a number of preparations, in most cases the mechanism underlying the response decrement is unknown. Several circuit models based on plausible theories of habituation have been constructed to help to interpret experimental results and to explore possible habituation mechanisms[2–5]; such models can help identify the requisite properties of decremental and incremental effects within the framework of a particular theory. With this in mind, I have explored, by mathematical analysis and computer simulation, a circuit model based on Thompson's two-process theory of habituation[5,6]. This theory was chosen as the basis of the work because of its wide applicability and acceptance in physiology and psychology. I show here how the effects of stimulus intensity can be realised by a circuit model similar to one proposed by Groves and Thompson[5].

Fig. 1 Curves of relative habituation in cat spinal cord, taken from ref. 6 with permission of the authors and publisher (their Fig. 7*a*). Stimulus strength: *a*, strong; *b*, moderate; *c*, weak.



In Thompson's two-process theory, response strength is determined by the sum of transmissions in two independent sets of channels from stimulus to response. Transmission decrements occur in direct stimulus–response channels, leading to observed response decrements at low stimulus intensities. Transmission increments, or sensitisations, occur at higher stimulus intensities in indirect channels associated with the overall state of reactivity of an organism. Dishabituation, then, is a transient facilitation superimposed on a depressed response channel following presentation of a novel, usually strong, stimulus. During habituation training, the balance between decrement and sensitisation produces a response that is stronger or weaker than the control (unhabituated) response. This balance shifts with the number of presentations at a given stimulus intensity to generate response curves of the form shown in Fig. 1, which were derived from experiments on the handlimb flexion reflex of spinal cats[6]. At low intensities the decrement prevails, whereas at higher intensities sensitisation causes the response to rise above its control level in early trials. Thompson[5] has illustrated the two-process theory with a circuit model which is proposed for cat spinal cord. In this circuit, stimulation results in synaptic depression in the direct stimulus–response path, and in synaptic increment or enhanced cell excitability in the indirect path, or state portion.

My model circuit (Fig. 2) is a simplification of Thompson's model. The circles represent cells or cell populations (referred to here simply as 'cells'). Cell H receives the external stimulus from its afferent, I. The output of cell O represents the circuit's overall response. The direct connection from H to O represents the direct stimulus–response channel. Cell S is the indirect path, or state system, of the circuit, and the channel from H to O through S represents the effect of this state system on the overall output. The output of each cell is a weighted sum of its inputs; that is

$$H = w_0 I \tag{1}$$

$$S = w_1 H \tag{2}$$

$$O = w_2 H + w_3 S \tag{3}$$

The value of each cell's output at a given moment may be interpreted either as the instantaneous firing rate of a single cell or as the number of cells in a population that are then firing. The value of the external stimulus may be thought of similarly. The value of each coupling weight may be regarded as the strength of the synapse connecting the two corresponding cells or as the effective synaptic transmission strength between two populations.

For the direct stimulus–response channel to become less effective with use, the coupling weight w_2 must become smaller on repeated stimulation. Similarly, the compound effect of w_1 and w_3 must increase with use to represent the sensitisation process. The influence of I on H does not change with stimulation, so w_0 is fixed. Changes in w_2 must occur so as to generate responses that yield a negative exponential function of the number of stimulus presentations. Further, the strengths of all coupling weights must tend towards their initial values in the absence of stimulation of the system. These requirements can all be met if each weight is modelled with a first-order differential equation which is driven by the output of the associated presynaptic cell. So

$$T_i \dot{w}_i = w_{i0} - w_i + a_i F_i(X); \quad i = 1, 2, 3 \tag{4}$$

where $\dot{w}_i$ denotes the time derivative of the associated weight value, and

$$X = \begin{matrix} H \text{ for } i = 1, 2 \\ S \text{ for } i = 3 \end{matrix} \tag{5}$$

For each i, w_{i0} is the initial value of the weight and the resting level towards which it moves when stimulation stops. The rate of return, which represents the rate of spontaneous recovery of habituation and sensitisation, is determined by the time constant, T_i. The functions F_i determine the way each weight is affected by its modifying input, X, and are non-negative for all values of X. If a_i is negative, the corresponding weight will be decreased by stimulation of its associated presynaptic cell; if a_i is positive, the weight will be increased.

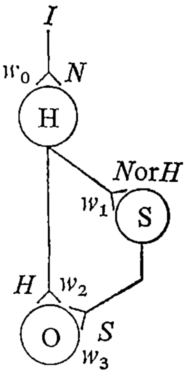


Fig. 2 Two-process circuit. N, non-plastic synapse; H, depleting synapse; S, sensitising synapse.

To determine the modification function for each weight, note first that sensitisation is a direct function of stimulus intensity[6], for now I suppose that $F_3(S)$ is simply S itself, so that the rate of increase of w_3 varies linearly with the output of cell S. Thompson maintains that relative habituation due to the decremental process alone is independent of stimulus intensity[6]. This requires that the F_i associated with each decreasing weight be a constant—that is, each weight that decreases with use must be driven by a constant at each presentation of a stimulus, regardless of stimulus intensity. More detailed circuits have been designed that obviate the need for this assumption, but in the investigations reported here I have used the circuit of Fig. 2 and the assumption of constant synaptic modification drive.

There are two ways that the circuit operating under these assumptions can generate curves of relative habituation similar to those in Fig. 1. They are based on whether or not w_1 is allowed to vary with use. If w_1 is fixed and w_3 changes more rapidly than w_2, at moderate and high stimulus intensities the overall response to repeated stimulation first increases across trials as w_3 increases, then falls as w_2 decreases. At low intensity, only the decrease in w_2 is significant, so the response only decreases across trials. Curves of relative habituation generated in computer simulation of this case are shown in Fig. 3. Note that in this case, since the output of cell S is constant across trials, sensitisation itself does not fall after rising, as required by Thompson's theory. The curves of Fig. 3 are qualitatively similar to those of Fig. 1, however, indicating that this assumption about sensitisation may not be required to generate such curves.

If w_1 is allowed to decrease with use, but more slowly than w_3 increases, sensitisation first increases, then decreases, across trials as the output of cell S decreases. This effect alone can generate response curves like those of Fig. 1. When added to the strategy in which changes in w_2 and w_3 generate the desired response curves, a small drop in w_1 causes the effect of sensitisation to be reduced further in later trials. This decrease in w_1 allows greater variation in w_3 to produce more sensitisation in early trials but with a return closer to control levels in later trials.

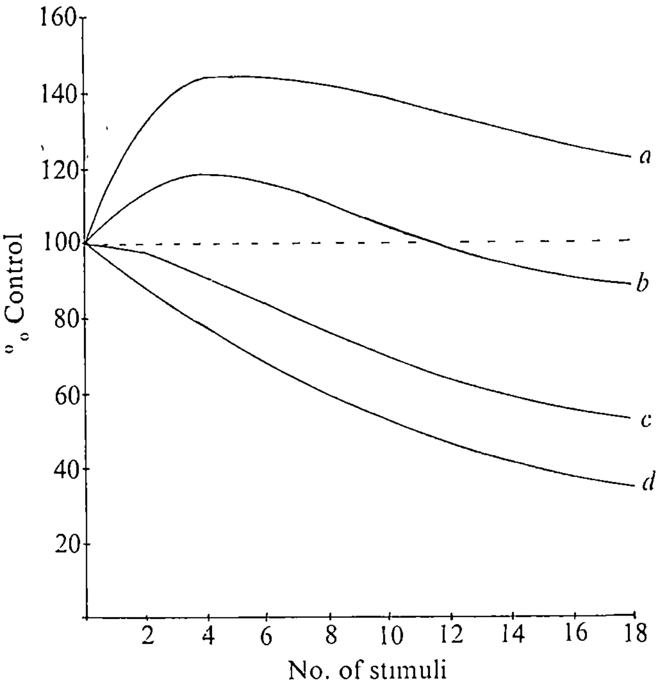


Fig. 3 Curves of relative habituation generated by computer simulation of the circuit of Fig. 2. Stimulus intensities: a, 8.0; b, 5.0; c, 2.0; d, 1.0.

Computer simulation of this simple habituation model has thus shown how the processes of decrement and sensitisation postulated in the two-process theory may vary with respect to one another to generate realistic response curves. Thompson's assumption that sensitisation decreases in later habituation trials has been shown to be unnecessary for the production of the intensity curves of Fig. 1. Such variation can lead to lower final response levels at higher stimulus intensities, however, and is necessary to explain habituation or dishabituation.

Further simulations have shown how the effects of stimulus frequency could arise in the model, and what effects nonlinear weight modifications could produce. A network using the circuit of Fig. 2 as a basic unit has also been simulated to investigate stimulus generalisation, and intensity-independent relative habituation has been produced by an augmented model that uses synaptic weights which decrease as a function of stimulus intensity. My simulations have provided insights into plausible functional organisations that may be difficult to investigate experimentally, and as such should contribute to the design of more decisive future experiments.

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Sprouting and regeneration of synaptic terminals in the frog cardiac ganglion

SYNAPTIC connections are capable of a remarkable proliferation after nervous tissue has been damaged and some synapses have been destroyed. This proliferation, or 'sprouting', of remaining, intact, synapses within partially denervated tissue has been found to occur in the peripheral, autonomic and central nervous systems[1–5]. As yet, however, the functional ability of these sprouted synaptic contacts has not been extensively tested and the persistence of the sprouted synapses over long periods has not been studied. We have been investigating these problems in the parasympathetic cardiac ganglion in the frog. Earlier studies have shown that when the nerve supply to the ganglion is partially destroyed by crushing one vagus nerve, axons in the other vagus nerve sprout and synapse with the denervated neurones[1]. Here I describe experiments in which the persistence of the sprouted synapses in the cardiac ganglion was tested by allowing the damaged axons to regenerate into the tissue. The results indicate that regenerating vagal fibres are able to re-establish some, but not all, of their synaptic connections and that innervation from the sprouted nerve decreases.

The cardiac ganglion in the frog is well suited for studying synaptic transmission at sprouting synapses. Because the ganglion is very thin, individual neurones can be seen in the living isolated preparation and this greatly facilitates intracellular microelectrode recording. Ganglion cells are grouped along two branches of the vagus nerves as they traverse the interatrial septum[1,6]. Each branch contains preganglionic axons from both left and right vagus nerves since the branches are joined by a chiasma near the entry of the vagus nerves into the heart. Ganglion cells are mostly innervated by the ipsilateral vagus nerve. For example, along the left branch of the ganglion in control animals, 87% of the neurones receive inputs from the left vagus and 48% from the right (about one-third of the cells are innervated by both nerves)[1]. Many ganglion cells are innervated by two or more preganglionic fibres; usually only one fibre produces suprathreshold excitatory postsynaptic potentials (e.p.s.p.s) and stimulation of the other fibres evokes subthreshold responses (Fig. 1*a*). After crushing the left vagus nerve, the remaining vagal axons sprout and within about 1 week, all the ganglion cells are innervated by the right vagus nerve[1]. The experiments described below were carried out at longer intervals after crushing the nerve, to study regeneration and the fate of the sprouted synapses.

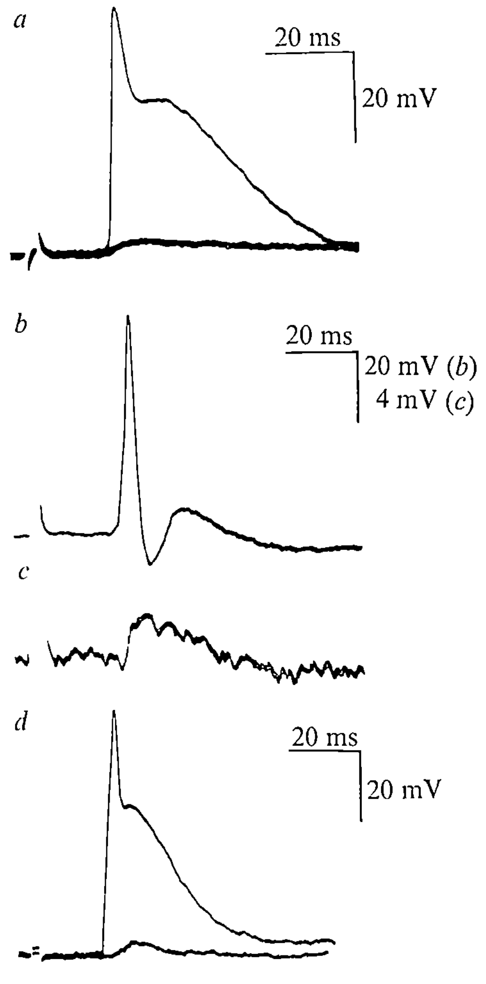
The experimental methods have been described previously[1] and will only be outlined briefly here. The left vagus nerve was crushed with fine forceps in frogs anaesthetised with 0.5% tricaine methanesulphonate (Sigma). The animals were allowed to recover for varying periods before synaptic transmission was tested. Neurones in the isolated cardiac ganglion were impaled with fine-tipped microelectrodes. E.p.s.p.s were recorded in response to stimulation of the right and/or left vagus nerves, which were drawn into separate suction electrodes.

As the left vagus nerve renegerated, it re-established some synaptic connections. E.p.s.p.s produced by regenerating fibres tended to be subthreshold early in the course of the regeneration (4–8 weeks), (Fig. 1, *b* and *c*). Later, both sub- and suprathreshold e.p.s.p.s were evoked by stimulation of regenerating fibres, as in normal ganglia (Fig. 1*d*). In addition, as the regenerating fibres re-established synaptic connections, there was a decline of innervation from the right vagus (Fig. 2). Experiments are in progress to determine whether a comparable decline of innervation from the right vagus nerve occurs when reinnervation is prevented.

At the longest recovery period studied, about 42 weeks, the innervation pattern of the ganglia was not restored to normal; the nerve supply to cells along the left branch of the ganglion was still dominated by axons from the right vagus nerve, in contrast with unoperated animals (Fig. 2). The regenerating fibres were unable to restore completely their original connections.

These data differ from the results of experiments in which the nerve supply regenerates to ganglion cells that are innervated by an implanted foreign nerve and not by sprouted synaptic terminals; Purves found that when the preganglionic supply to sympathetic ganglia (superior cervical ganglia) of guinea pigs is cut and the parasympathetic vagus nerve is rerouted into the ganglia, the vagal endings form synaptic contacts which are not displaced when the original innervation regenerates[7]. That is,

Fig. 1 Intracellular recordings from multiply-innervated parasympathetic neurones located on the left branch of the cardiac ganglion. *a*, Superimposed recordings from a ganglion cell in an unoperated animal Threshold stimulation of the left vagus nerve evoked a subthreshold e.p.s.p. Stronger stimulation produced a suprathreshold response. *b* and *c*, Ganglion cell innervated by both the right vagus (*b*) and regenerated left vagus nerve (*c*) Stimulation of the left vagus produced a subthreshold e.p.s.p. The left vagus nerve had been crushed 41 d before the experiment. *d*, Ganglion cell with multiple innervation from the regenerated left vagus nerve. The left vagus had been crushed 302 d before the experiment. Weak stimulation of the regenerated nerve evoked a subthreshold e.p.s.p. Stronger stimulation produced a suprathreshold response, as in unoperated ganglia.



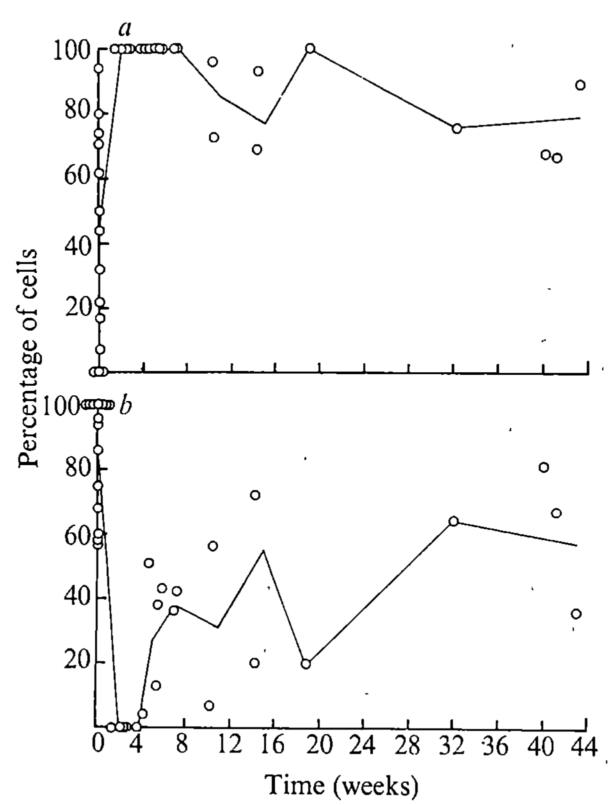


Fig. 2 Sprouting and regeneration of synapses in the cardiac ganglion. Each graph shows the percentage of cells located on the left branch of the ganglion which were innervated by the right (*a*) and left (*b*) vagus nerve at different periods after the left vagus nerve was crushed. Each circle represents data from one ganglion (15–30 cells recorded per ganglion). Circles on the ordinate show data from unoperated ganglia. A line has been drawn connecting the averages of data grouped into weekly intervals; the last three points were grouped together. Sprouting of synapses from the right vagus nerve is indicated by the increase of right vagal innervation from 48 to 100% within 1 week of the operation (*a*). Regeneration of left vagal synapses begins after about 4 weeks (*b*). At the first stages of regeneration (4–8 weeks) there did not seem to be any change in right vagal innervation (*a*), but this is misleading; slight decreases in the size of right vagal e.p.s.p.s are not indicated on this figure.

many of the sympathetic ganglion cells are innervated by both nerves. On the other hand, the present experiments confirm and extend to a cellular level the observations of Guth and Bernstein[8] who studied the reinnervation of superior cervical ganglia of cats after partial deafferentation. They reported that after damaging thoracic sympathetic rami which contributed pupillodilator fibres, remaining intact preganglionic fibres sprouted and took over this function. When the preganglionic fibres regenerated, stimulation of the sprouted nerve no longer produced pupillodilation; reinnervation by the appropriate axons was accompanied by a decrease in the innervation arising from sprouted fibres.

The present experiments do not explain the mechanism by which innervation from the sprouted right vagus nerve decreases during regeneration of the left vagus nerve or even if the reinnervation causes this decrease. Because transmission at the sprouted synapses is indistinguishable from normal synapses[1], it is not possible to determine whether sprouted or original right vagal terminals are lost during regeneration. It is possible that right vagal synapses are 'suppressed' by the regenerating axons but are left structurally intact[9]. It is also possible, however, that a slow rate of spontaneous synaptic degeneration occurs[10] and when a right vagal ending degenerate, axons from the left and right vagus nerve compete for the vacated site.

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Function of muscarinic and nicotinic acetylcholine receptors

ACETYLCHOLINE receptors in vertebrates can be classified in two categories—nicotinic and muscarinic—on the basis of differential sensitivity to agonist and antagonist compounds[1,2]. This pharmacological distinction, although useful both in therapeutics and in experimental physiology, has no known biological significance. I wish to show here that there are marked kinetic differences between nicotinic and muscarinic receptors, and to suggest that these differences have a clear physiological meaning.

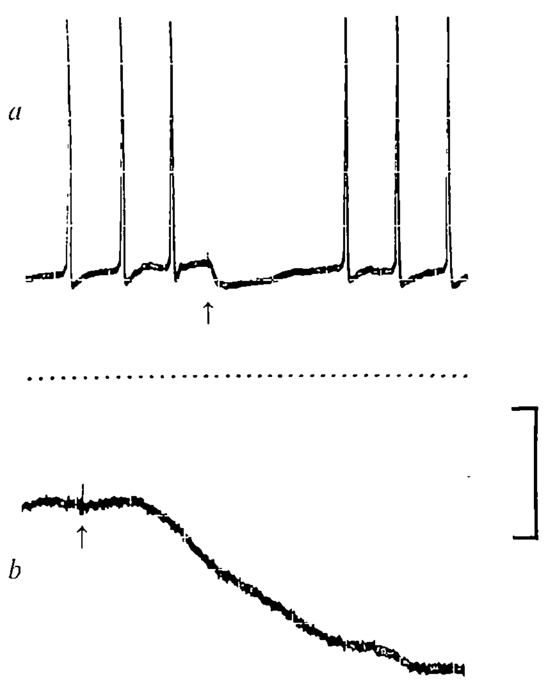
Two features are shared by all nerve-mediated muscarinic responses that have been studied electrophysiologically: the minimum latency is ~ 100 ms, and the duration after a single stimulus is 0.5 s or longer[3–7]. In contrast, however, the synaptic delay at the skeletal neuromuscular junction may be only 0.22 ms (ref. 8), and even at the slowest nicotinic synapses, the synaptic potential lasts no more than 30–100 ms (refs 9 and 10). The discrepancy in the latencies cannot be explained by morphological differences between nicotinic and muscarinic junctions, although the separation of nerve and effector is more variable at the autonomic junctions than at the skeletal neuromuscular junction[11,12]. Experiments in which acetylcholine (ACh) was applied iontophoretically to muscarinic receptors of cultured intestinal smooth muscle cells have shown that the long latency is post-junctional in origin[13]. This result has been confirmed in adult smooth muscle[14] and in salivary glands[15].

One circumstance in which a long slow response is of obvious importance is during vagal slowing of the cardiac pacemaker. This function would be ill served by an inhibitory mechanism operating at 'nicotinic speeds', for not only would most of the inhibitory input be ineffective, but the interval between beats would be critically dependent on the arrival of inhibitory impulses immediately before the projected time of discharge. There is little direct evidence that cardiac muscarinic receptors operate slowly, since estimates of the latency after single volleys have been made using preganglionic stimulation[3,4,16]. The delay due to relay in the cardiac ganglia, however, is probably small[17].

del Castillo and Katz[4] applied ACh iontophoretically to the sinus venosus of the frog, and could duplicate the main features of the response to vagal stimulation. I have examined the onset of the response to close iontophoretic application of ACh in a preparation (interatrial septum of the frog *Rana temporaria*) which allowed observation of the position of micropipettes with good phase contrast optics at a magnification of ×140 or ×220. The minimum latencies of intracellularly recorded responses (Fig. 1) were 200–300 ms at 24 °C and 400–550 ms at 15 °C. The minimum latency was affected little by lateral displacements (up to 100 μm) of the ACh pipette away from the recording site. It was also relatively insensitive to small (5–15 μm) vertical displacements. This behaviour parallels that seen in comparable experiments on smooth muscle[13]. Ganglion-blocking drugs (hexamethonium, 2×10^{-4} M, mecamylamine, 10^{-4} M) had no effect on the latencies. A striking feature of the responses was their long duration, brief pulses of 0.5–1 nC producing hyperpolarisation which could last for 8 s or more.

The effective stimulus presented to the tissue can be estimated from the diffusion equations, if some simplifying assumptions are made about the geometry. For example, ACh diffusing freely from a point source 10 ms in duration situated 10 μm above an infinite plane surface will reach its peak concentration immediately below the source in less than 30 ms. The concentration decline is also rapid, reaching 5% of the peak value by 400 ms. Since the muscle cells are interconnected electrically[18], ACh acting at some distance from the recording site presumably also contributes. The average concentration, calculated as the concentration-area product integrated over the whole plane, is perhaps a fairer measure (Fig. 2). The onset of the response lags behind the stimulus. The effective diffusion distances, which might be longer than suggested by the model, are uncertain and there may be diffusion barriers. But the minimum distance between the ACh pipette and the muscle was certainly less than the value of 10 μm assumed for calculation, since in many experiments the tip of the ACh pipette could be seen to dimple the surface of an underlying muscle cell. These results support the conclusion that inhibitory muscarinic responses in the heart are at least as slow as excitatory muscarinic responses elsewhere.

Fig. 1 Intracellular electrical records from isolated interatrial septum of the frog, showing responses to iontophoretically applied ACh. *a*, Record from a spontaneously active preparation. The arrow shows the time of application of a 15-ms, 40-nA pulse of ACh. *b*, Initial phase of response in quiescent preparation at higher amplification and sweep speed, showing well defined latency. Temperature 15 °C. ACh pulse (10 ms, 70 nA) at arrow. Vertical calibration 50 mV in (*a*), 5 mV in (*b*). Time marker, 1 s in (*a*), 0 1 s in (*b*).



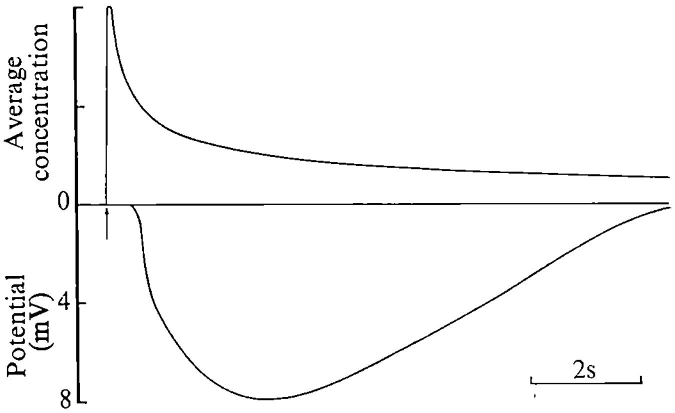


Fig. 2 Comparison of redrawn voltage response (lower curve) with calculated average concentration change of ACh Upper curve calculated as $M(\pi Dt)^{-\frac{1}{2}} \exp(-x^2/4Dt)$, where M is amount released from instantaneous point source, D is the diffusion constant (8.10^{-6} cm^2 s^{-1}), x is the vertical separation between source and tissue (10 μm) and t is time. Vertical scale arbitrary. The expression is identical with that giving the concentration at distance x from a plane source. The error made in assuming instantaneous release in place of a 10-ms source is negligible at times greater than ~ 50 ms.

The duration of the inhibition after a single vagal volley is 20 s in the turtle[16], 5–7 s in the frog[4] and 2–3 s in the cat[3]. Thus a single impulse has effects which extend over several cardiac cycles, facilitating temporal summation and continuity of successive responses, even at the low firing frequencies of autonomic nerves. It is probably no coincidence that animals with low heart rates show longer vagal responses. Conversely, at the skeletal neuromuscular junction it would be inappropriate to have provision for temporal summation, since smooth grading of contractions is obtained by other means. Nicotinic receptors provide the required 1 : 1 transmission with minimum latency and maximum temporal precision. If the argument given earlier is valid, the excitation produced by adrenergic nerves in the heart should be slow. This is indeed the case: the latency of sympathetic β cardioacceleration is several seconds and the effects of a short period of stimulation last for minutes[19,20]. Responses of excitatory α receptors, for instance in the vas deferens, are some orders of magnitude faster[21].

How are the slow responses mediated? There are indications that cyclic GMP acts as an intracellular 'second messenger' for muscarinic receptors[22–24], and there is strong evidence that cyclic AMP plays the same part for β receptors[23,25]. The evidence from latencies and time courses is not at variance with these ideas.

I propose that muscarinic receptors are specialised to allow temporal summation and thus continuity of action, whereas nicotinic receptors are specialised to prevent it. The long latency, as such, at muscarinic junctions is of doubtful biological relevance. Nevertheless, it provides a convenient pointer to a physiologically significant slowness of response.

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Glutamate and quisqualate noise in voltage-clamped locust muscle fibres

GLUTAMATE-SENSITIVE receptors occur widely, both in vertebrate and invertebrate nerve and muscle membranes[1,2]. Information concerning the action of agonists on glutamate receptors and the kinetics of the associated ion channels may be obtained from a study of the noise which results from conductance fluctuations during drug action[3–8]. Analysis of voltage-clamped noise has been used in the present work to investigate the lifetime of an open channel in the presence of a steady concentration of a chemo-excitant, and to obtain an estimate of the single-channel conductance. In addition, we have examined the dependence of the channel duration on temperature and membrane potential and have compared the channel kinetics produced by different agonists and by the neurally released transmitter.

The metathoracic extensor tibiae of adult locusts (*Schistocerca gregaria*) was used[9] because its junctional glutamate receptors are easily accessible[10], and also because its fibres, being only ~1.5 mm long with a diameter of 100 μm, have a high input resistance in Cl-free medium (5–8 MΩ) and long space constant (1 cm), which makes them very suitable for voltage-clamp studies. Voltage recording and clamping microelectrodes were inserted within 100 μm of each other in the middle region of a fibre. L-Glutamate (Na salt *p*H 8; 0.1 or 1.0 M) or quisqualate (Na salt *p*H 8; 0.1 M) was applied iontophoretically from a micropipette (50–200 MΩ) positioned between recording and clamping microelectrodes, and at a sufficient distance from the junctional regions to produce a gradual increase in drug concentration at the junction and so reduce effects of fluctuations in ejection rate.

In locust muscle there are at least two types of glutamate-sensitive receptors[11]. The activation of one type (extra-junctional D and excitatory junctional receptors) leads to membrane depolarisation caused by the opening of channels which are permeable mainly to Na and K ions[10]. Activation of the second type (H receptors) produces membrane hyperpolarisation due to the opening of Cl channels. To avoid complications which would result from simultaneous activation of both membrane channels, we abolished the hyperpolarising effect by removing Cl ions from the medium[11].

The efficacy of clamping was evaluated from the residual voltage change recorded by a third intracellular microelectrode inserted at various positions along the fibres. Iontophoretic application of glutamate to the muscle membrane produced current fluctuations above the background. The variance of this glutamate noise increased as a linear function of the mean membrane current (μ_I). The energy spectra of the conductance fluctuations follows a $1/f^2$ curve (Fig. 1*a* and *b*) which fits well the theoretical relationship

$$S(f) = \frac{\gamma\mu_I(V_m - V_{eq})/\pi f_c}{1+(f/f_c)^2} \ (\mathrm{A^2\,s}) \qquad (1)$$

developed previously for the relaxation of acetylcholine (ACh)-controlled two-state channels in frog endplate[4] where f = frequency (Hz); f_c = cutoff (half-power) frequency = $1/2\pi\tau$ (where τ is the mean lifetime); γ is the conductance of a single

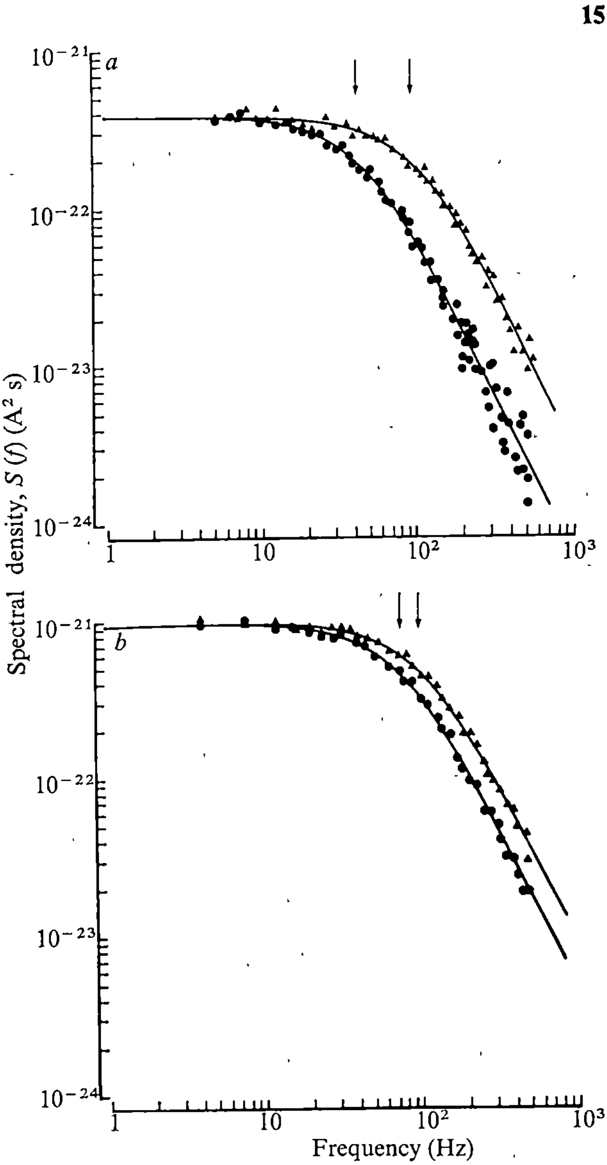


Fig. 1 Energy spectra of glutamate current noise obtained in voltage-clamped muscle fibres. *a*, Temperature dependence of the glutamate noise spectra from a single fibre at V_m = 104 mV. ●, 8.3 °C; ▲, 22.5 °C. The half-power frequencies are: f_c = 98 Hz at 22.5 °C and 45 Hz at 8.3 °C, for mean currents of 11 and 9 nA respectively. Vertical axis may be converted to conductances by multiplying ordinate values by 92.46. Single-channel conductances calculated from the fitted curves are $\gamma = 1.03\times10^{-10}$ mhos at 22.5 °C and 3.91×10^{-11} mhos at 8.3 °C. For comparison, the curve at 8.3 °C has been scaled by multiplication (simple vertical translation on log–log coordinates) by 1.5. Arrows mark half-power frequencies (R_{in} = 7.5 MΩ). *b*, Membrane potential dependence of glutamate noise spectra from a single fibre (different from that shown in *a*) at T = 23.0 °C. ●, 60 mV; ▲, 100 mV. Curves fitted to the data have f_c = 100 Hz and $\gamma = 2.54\times10^{-10}$ mhos at 100 mV, and f_c = 76 Hz and $\gamma = 2.34\times10^{-10}$ mhos at 60 mV. μ_I = 12 nA at 100 mV and 16 nA at 60 mV. Vertical axis may be converted to conductances by multiplying ordinate values by $(V_m - V_{eq})^{-2}$. Curve at 100 mV scaled by 0.97 for direct comparison of the cutoff frequencies. Arrows mark half-power frequencies (R_{in} = 5.0 MΩ). All spectra shown are from noise records taken during steady local application of glutamate. Data were edited to eliminate spontaneous miniatures. Membrane current was recorded on analog tape (bandwidth 1–2 5 Hz) through an active virtual earth and digitalised (32,768 points at 4,096 Hz sample rate) after low pass filtering (500 Hz, $1/f^8$). The Fourier transform (FFT algorithm) was calculated for 1,024 point segments and averaged after Hann filtering. Spectral values, equally spaced in frequency, are shown on log–log coordinates. No averaging across frequencies was performed. Data were processed with a Linc-8 in double precision floating arithmetic. Control noise spectra (1.25×10^{-23} A² s at 20 Hz) taken immediately before application of glutamate are subtracted point by point to remove background contributions from non-receptor membrane currents and electronics. Clamp quality at the voltage electrode was better than 99.8% at 0 Hz and better than 90% at 1.6 kHz over the entire membrane potential range used. Curves represent the relationship in equation (1).

open ionic channel; V_m = membrane potential and V_{eq} = equilibrium potential for glutamate, taken throughout as zero mV (ref. 11).

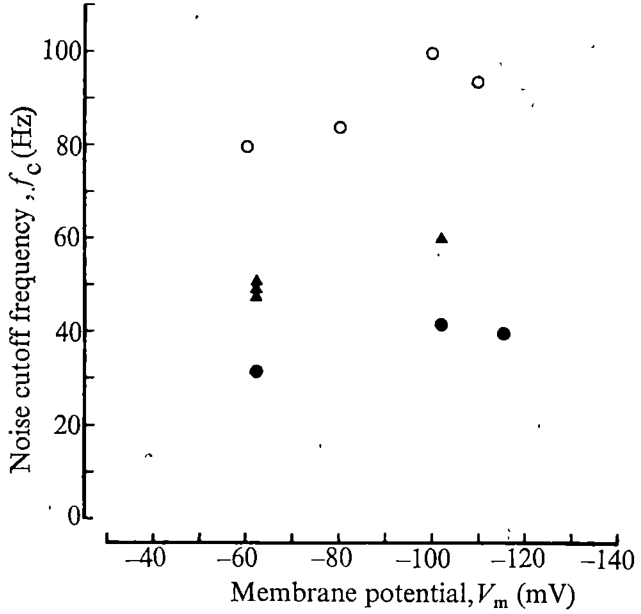
Although we positioned the electrodes near a junctional region, the glutamate presumably activated extrajunctional receptors as well. The fact that the noise spectra had a single $1/f^2$ component suggests that the contribution from extrajunctional sites is small, or that the kinetic properties of junctional and extrajunctional channels are very similar. This conclusion is supported by the fact that spectra of current fluctuations obtained during application of glutamate in a bathing solution agreed well with those obtained during iontophoretic application.

As shown in Fig. 1*a*, decreased temperature prolongs the open state of the glutamate-produced channel from 1.59 ms at 22.5 °C to 3.23 ms at 8.5 °C, giving an apparent single barrier activation energy of $E_A = 8.37$ kcalorie M^{-1} as calculated from the Arrhenius equation. The resulting Q_{10} of 1.45, is lower than that observed for ACh receptor channels[4]. Lowering the temperature has been shown to increase the value of τ derived from glutamate noise in squid nerve cells[6].

The mean value of the conductance of the single channel opened by glutamate was $\gamma = 2.31 \times 10^{-10}$ mhos at 23 °C, varying in different fibres from 1.0×10^{-10} to 2.6×10^{-10} mhos. In contrast with ACh sensitive channels[4], the conductance of the single channels opened by glutamate changes with temperature (Fig. 1*a*). By fitting equation (1) to the measured noise spectra, it was found that γ decreases as the temperature is lowered: in the fibre illustrated in Fig. 1*a*, $\gamma = 1.03 \times 10^{-10}$ mhos at 22.5 °C and 0.39×10^{-10} mhos at 8.5 °C for values obtained at the same site (*t* test for difference, $P > 0.01$). This temperature dependence ($Q_{10} = 2.12$) is greater than that of the intra- or extracellular conductivity (Q_{10} = 1.37 (ref. 12) and 1.26 (ref. 13), respectively). γ showed no significant correlation with V_m or μ_I.

Glutamate noise spectra change with membrane potential (Fig. 1*b*). The duration of an open single channel decreases with hyperpolarisation of the membrane ($\tau = 2.12$ at -50 mV and $\tau = 1.56$ at -110 mV at 23 °C). Figure 2 summarises this relationship for three cells over a 65-mV range of potentials at two temperatures. The voltage dependence of the half-power frequency has a high statistical significance at all temperatures investigated.

Fig. 2 Membrane potential dependence of the cutoff frequency for the energy spectra of glutamate noise. Results shown are from three different cells: ●, 8.0 °C; ▲, 8.5 °C; ○, 23 °C. Spectra were fitted to equation (1) and the cutoff frequencies determined. In terms of the model discussed, the average single-channel lifetime at each V_m and T may be calculated as $1/2\pi f_c$ (s).



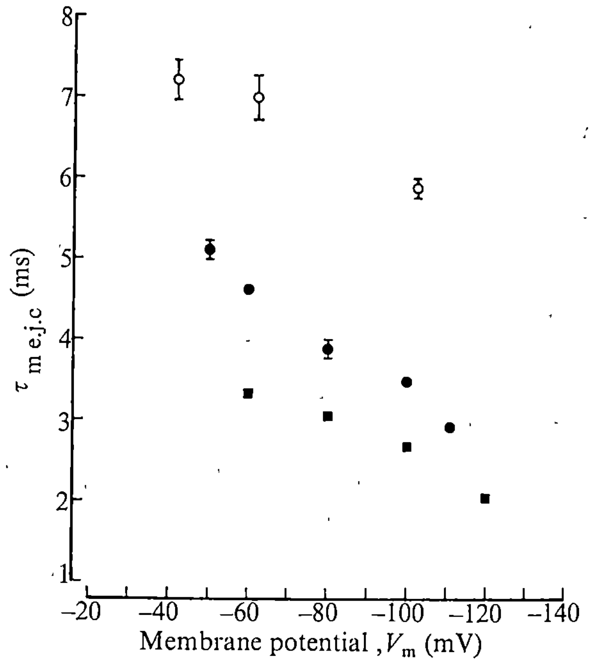


Fig. 3 Membrane potential dependence of the time constant of decay of spontaneous m.e.j.c.s. Results shown are from three different cells: ●, 20 °C; ■, 23 °C; ○ at 9 °C. Each event was digitalised at 5 kHz and the decay phase plotted on log–linear coordinates. The straight line through these points was fitted by the least squares method to give the time constant of the m.e.j.c. Approximately 100 such values were determined at each V_m and T. Histograms of the time constants used in the figure were distributed normally about the mean. The standard deviations ranged from 1.25 ms at 50 mV to 0.38 above 100 mV. *t* Tests for the confidence of the voltage dependence gave > 0.01 for a 60-mV change and between 0.15 at low V_m and 0.01 at 100 mV for a 20-mV step. The voltage residual during clamping was always less than 5%. Input resistances varied between 1 MΩ at 50 mV and 7.5 MΩ at 100 mV. All values are means±s.e. (shown only when greater than the size of the symbols). The larger s.e. obtained at low temperature result from the reduction in γ and the low frequency of m.e.j.c.s.

Quisqualic acid, a glutamate analogue derived from the seed of *Quisqualis indica*, is several times more effective than glutamate on glutamate receptors of crayfish muscle[14] and rat spinal neurones[15]. As the activation of ACh receptors by different drugs produces noise with different characteristics in frog muscle[16], it was of interest to compare the noise resulting from quisqualate and glutamate action in locust.

Iontophoretic application of quisqualate to muscle fibres produced a pronounced increase in μ_I and in current noise. The energy spectra of the current fluctuations during a steady level of quisqualate action can be described by equation (1) with f_c = 45 Hz at 23 °C and -116 mV. In an experiment in which the actions of glutamate and quisqualate were compared at the same sites, the lifetime of the quisqualate-produced channel (τ = 3.54 ms) was 1.8 times that obtained with glutamate. The conductance of the single channels produced by quisqualate ($\gamma = 2.42 \times 10^{-10} \pm 0.25$ mhos, $n = 10$, at 23 °C) and glutamate ($\gamma = 2.32 \times 10^{-10} \pm 0.12$ mhos, $n = 4$, at 23 °C) were, however, almost identical (*t* test, $P > 0.01$). In addition, quisqualate noise showed membrane potential dependence.

If glutamate is the natural transmitter, and provided the transmitter concentration during a miniature potential subsides very quickly, compared with the life span of an open channel, then the time constant of decay of the spontaneous miniature excitatory junction current (m.e.j.c.), $\tau_{m.e.j.c.}$ should approximate closely to the value of τ derived from glutamate noise (τ_{noise})[4]. Furthermore, both time constants should then display similar voltage and temperature dependence. Figure 3 shows such a relationship for $\tau_{m.e.j.c.}$ which approximately doubles for a 70-mV change in V_m or a 15 °C change in temperature (*t* test for both, $P > 0.05$).

There are conflicting reports on the voltage dependence of nerve-evoked excitatory junctional currents at glutamate-sensitive synapses[17–19]. Our results are in agreement with those previously reported for the crayfish nerve–muscle junction[18].

Time courses for m.e.j.c.s varied widely in all fibres, a finding confirmed by focal extracellular recording. The mean value of $\tau_{\mathrm{m.e.j.c.}}$ estimated from histograms obtained at different voltages and temperatures is longer than the open single-channel lifetime, as derived from the glutamate noise. Nevertheless, a proportion of the m.e.j.c.s, recorded both intra- and extracellularly at each site, gave τ values approximately the same as those of τ_{noise}. This point was checked further by simultaneous recording of focal extracellular miniature potentials and clamped intracellular miniature currents, allowing direct comparison of decay time constants of the same event. Both recordings gave similar τ values. A number of factors could contribute to the prolongation of $\tau_{\mathrm{m.e.j.c}}$, relative to the single-channel duration. These include: barriers to diffusion of transmitter across and out of the cleft; 'compression artefact'[20]; non-uniform space clamp; synaptic membrane areas with receptors possessing different relaxation times. These possibilities are being investigated.

It can be concluded that glutamate receptors, which mediate depolarisation in locust muscle, open channels with a mean lifetime which depends on temperature, membrane potential and agonist, while the single-channel conductance is dependent on temperature. The increase in conductance of the single channel with increased temperature and the decrease in mean lifetime of the channel with hyperpolarisation, are in marked contrast to the endplate channels opened by ACh. In terms of a dipole model of the glutamate receptor–channel complex, the dipole component parallel to the electric field in the membrane has a polarity opposite to that of the ACh receptor in vertebrate muscle.

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Paradoxical response to amphetamine in developing rats treated with 6-hydroxydopamine

THE clinical syndrome of minimal brain dysfunction (MBD) in children is characterised by hyperactive motor behaviour, decreased attention span, impulsiveness and a variety of cognitive and perceptual problems. A striking feature of the disorder is the unusual response to stimulant medication. Thus, administration of amphetamine to children with MBD results in a sharp decrease in motor activity. Since the usual pharmacological response to amphetamine is an increase in motor activity, this response has been termed "paradoxical"[1]. We have described an experimental model in the developing rat that is strikingly similar to the clinical syndrome of MBD found in children[2]. Rat pups treated with 6-hydroxydopamine (6-OHDA) at 5 d of age develop increased motor activity and demonstrate cognitive difficulties in shuttle-box learning between 2 and 4 weeks of age. We now report that administration of (+)-amphetamine reduces the hyperactivity in 6-OHDA-treated rat pups, an effect paralleling the paradoxical response to this agent in children with MBD. The similarity of many of the cardinal features of MBD in an experimental model produced by depletion of brain dopamine in developing rats supports the belief that brain monoamines may be involved in the pathogenesis of this disorder.

All rat pups in a litter were randomly allocated as hyperactive or control animals at 5 d of age. Experimental hyperactivity was effected by intraperitoneal administration of desmethylimipramine (20 mg kg^{-1}) followed after 1 h by intracisternal 6-OHDA (100 µg 6-OHDA·HBr calculated as the free salt in 25 µl sterile saline)[2–5]. Control littermates received similar amounts of sterile saline intraperitoneally and intracisternally. Groups of 6-OHDA-treated and control rats were subsequently treated with saline or amphetamine (at doses of 0.25, 0.5, 1.0 and 5.0 mg kg^{-1}) 30 min before monitoring activity and activity was determined for a 60-min interval at 8, 12, 15, 19, 22 and 26 d of age using the following time-sample method[2,6,7]. Rats were randomised and placed in transparent Plexiglas cages, containing a water bottle and food, which were arranged in three rows on a steel cage rack. At the beginning of each minute the cages were scanned and a written note made of the behaviour of each rat at that time using one of the following categories· inactive—standing or sitting motionless; ambulating—walking or running about the cage; climbing—forepaws on the side of the cage or on water bottle; rearing—both forepaws clear of the floor; eating—gnawing at food; drinking—mouth in contact with water bottle; sniffing—sniffing at any part of the cage or in the air; grooming—any self-washing or licking movements; scratching—scratching body with hind leg. Any cumulative effect of (+)-amphetamine was minimised by the 3–4-d interval between administration of the agent. All measure-

Fig. 1 Effect of amphetamine on activity in control and 6-OHDA-treated rat pups. At 5 d of age animals received intracisternal injections of 25 µl saline or 100 µg 6-OHDA after pretreatment with desmethylimipramine. Activity was determined 30 min after injection of 0.5 mg kg^{-1} amphetamine throughout the first month of life. Activity is represented as mean ± s.e.m. First open column, control saline; second open column, control amphetamine; hatched column, 6-OHDA saline; solid column, 6-OHDA amphetamine. *Differs from saline, $P < 0.001$; †differs from saline, $P < 0.05$.

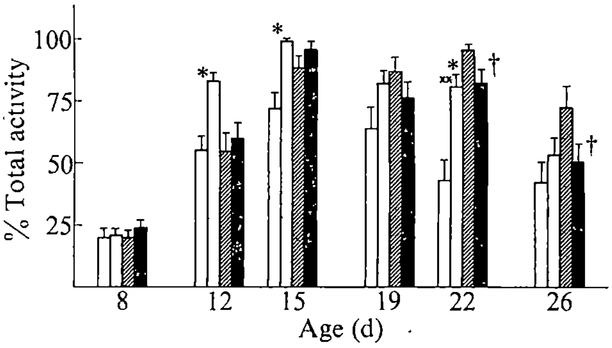

Table 1 Brain catecholamine concentrations

	Neonatal treatment			
	Vehicle		6-OHDA	
Dose amphetamine (mg kg^{-1})	Saline	Amphetamine	Saline	Amphetamine
0.25	(5)	(5)	(5)	(6)
Dopamine	532±25.1	520±36.0	114±43.2*	137±3.75*
Noradrenaline	267±11.0	265±20.5	246±8.49	241±17.4
0.5	(6)	(6)	(5)	(6)
Dopamine	488±28.9	480±17.3	191±33.4*	164±12 8*
Noradrenaline	345±27.1	278±11.0	313±23.5	283±17.7
1.0	(5)	(6)	(6)	(6)
Dopamine	573±65.2	478±23.5	299±27.6†	122±25.0*
Noradrenaline	275±20.6	255±5.6	272±28.3	250±17.4
5.0	(5)	(6)	(5)	(5)
Dopamine	570±16.6	586±37.2	163±64.1‡	143±16.6‡
Noradrenaline	252±11.6	193±8.23	232±13.0	209±26.7

*6-OHDA (saline or amphetamine) compared with vehicle (saline and amphetamine) $P<0.001$; †$P<0.01$; ‡$P<0.05$ (t test).

Dopamine and noradrenaline are means, ng per g wet weight±s.e.m. 6-OHDA (100 µg) or vehicle administered at 5 d of age. Amphetamine or saline administered at 8, 12, 15, 19, 22 and 26 d of age. Rats killed at 33 d of age. Number of rat pups given in parentheses.

ments were made between 1300 and 1600 to minimise the variation resulting from circadian periodicity. Total activity, ambulation and sniffing activity determinations in each group were subjected to analysis of variance. Rats were killed by decapitation at 33 d of age, their brains were removed rapidly and frozen at −80° C, and analysed within 2 weeks for dopamine and noradrenaline using a fluorimetric assay[8,9]. At each particular dose 6–8 animals were used in either control or 6-OHDA-treated groups. Differences in catecholamine concentrations between groups were analysed using t test[10].

The intracisternal administration of 6-OHDA to 5-d-old rat pups resulted in significant and comparable reductions in brain dopamine in both amphetamine- and saline-treated animals (Table 1). These reductions in dopamine concentration ranged from 21–39% of control values reflecting the variable amount of 6-OHDA actually reaching dopaminergic terminals by way of the cisternal route, and were in all cases significantly lower than their comparable littermates treated with vehicle solution. This contrasts with noradrenaline concentrations, which ranged from 91–108% of controls and were in no instances significantly reduced compared with the controls.

Total activity was significantly greater in 6-OHDA-treated rats between 2–4 weeks of age ($P<0.001$) than in littermate controls, confirming results of our previous investigation[2]. This increase in activity was primarily the result of significant increases in both ambulation ($P<0.001$) and sniffing ($P<0.001$) that occurred during this period.

Administration of amphetamine at doses of 0.25, 0.5 and 1.0 mg kg^{-1} to normal developing rat pups resulted in significant increases in activity from 12 d of age, and as early as 8 d in rat pups given 5.0 mg kg^{-1}. This increased activity was due to both increased ambulation and sniffing activity.

In contrast, administration of amphetamine between 2 and 4 weeks of age to 6-OHDA-treated rat pups resulted in a significant reduction in total activity (Fig. 1). This reduction occurred primarily at the expense of ambulatory activity, which was markedly reduced. Sniffing and other stereotyped behaviour did not change. Analysis of variance demonstrated the amphetamine-induced reduction in activity in 6-OHDA-treated rat pups at amphetamine doses of 0.5 mg kg^{-1} ($F=5.83$, d.f. 1, 370, $P<0.05$), 1.0 mg kg^{-1} ($F=6.10$, d.f. 1, 359, $P<0.05$) and 5.0 mg kg^{-1} ($F=6.49$, d.f. 1, 360, $P<0.05$). (+)-Amphetamine at a dose of 0.25 mg kg^{-1} had no significant effect on activity ($F=1.4$, d.f. 1, 320, $P>0.05$). There was significant correlation between amphetamine dosage and age ($F=6.38$, d.f. 16, 2,265, $P<0.001$) as well as between amphetamine dosage and 6-OHDA or saline treatment ($F=6.35$, d.f. 4, 2,265, $P<0.001$).

Our data indicate that, in certain conditions, administration of amphetamine results in a decrease in locomotor activity rather than an increase. Such a paradoxical response to amphetamine may be explained most parsimoniously by considering the relationship between amphetamine and brain monoamines. Although their exact nature is unclear, it is generally believed that the stimulant actions of amphetamine are mediated by way of central catecholaminergic mechanisms. Thus, amphetamine is known to increase the amount of dopamine and noradrenaline at the synaptic cleft by increasing granular release and by decreasing neuronal uptake[11–15]. Controversy exists over whether dopamine, noradrenaline, or both monoamines are responsible for the behavioural effects of amphetamine. The issue is complicated still further by the presence of two well documented dopaminergic systems within the brain. Recent evidence indicates that the mesolimbic dopaminergic pathway is essential for amphetamine-induced locomotor activity, whereas the nigroneostriatal dopaminergic system seems to mediate amphetamine-induced stereotyped activity[16–18].

We suggest that both the hyperactivity and the paradoxical response to amphetamine found in 6-OHDA-treated rat pups may be explained by considering that the nigroneostriatal dopaminergic system acts as a modulator of excitatory activity. This excitatory activity may be mediated by way of the noradrenergic pathways or by the mesolimbic dopaminergic system. Administration of 6-OHDA affects predominantly the nigroneostriatal dopaminergic pathway and thus excitatory activity, whether noradrenergic or mesolimbic dopaminergic occurs unrestrained. The denervated receptors in the dopaminergic inhibitory system are, however, supersensitive and so respond to a greater extent to catecholamines released presynaptically by amphetamine than do the undamaged receptors in excitatory pathways, leading to a predominance of inhibitory mechanisms and thus reduced activity.

That such a supersensitivity phenomenon does indeed exist may be demonstrated both behaviourally and pharmacologically. Administration of the dopaminergic stimulating agents apomorphine and L-dopa enhance both the locomotor and stereotypy response to amphetamine in adult rats treated with 6-OHDA (refs 19, 20). Furthermore, recent evidence suggests that the postsynaptic action of dopamine is mediated by way of a cyclic AMP-dependent mechanism. This adenylate cyclase response is significantly increased after denervation of striatal tissue, the presumed pharmacological correlate of supersensitivity[21–24]. The parallel between the syndrome produced in developing rats by depletion of brain dopamine and the clinical syndrome, together with the suggestion of reduced turnover of brain dopamine found in a small homogeneous group of children with the disorder[25], suggests that brain monoamines may play a role in the pathogenesis of MBD.

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Lesions of ascending dopaminergic pathways decrease forebrain glucose uptake

WE describe the changes in forebrain glucose uptake following various lesions of ascending catecholaminergic pathways. These pathways include dopaminergic fibres arising from the pars compacta of the substantia nigra (A9 of Dahlström and Fuxe[1]) and from other cell bodies in the ventral tegmentum (A8, A10) and noradrenergic fibres originating from the locus coeruleus (A6) and from other cell bodies in the midbrain (A7) and medulla (A1, A2, A5)[2]. We have found that destruction of dopaminergic fibres results in decreased forebrain glucose uptake.

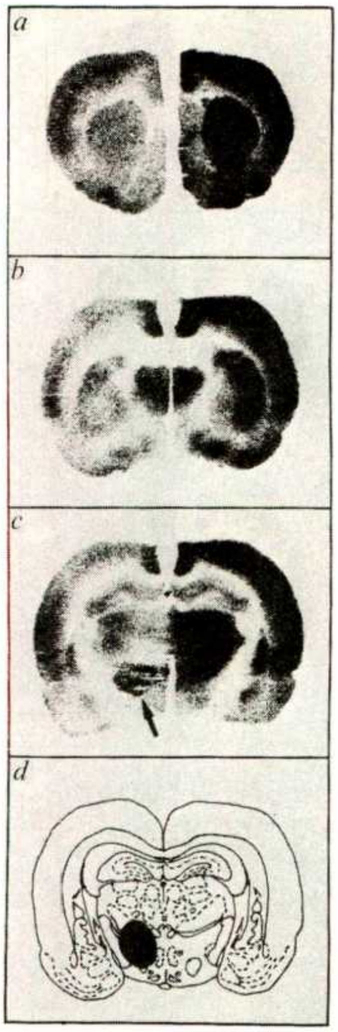


Fig. 1 ^{14}C-2-deoxy-D-glucose X-ray film autoradiographs of coronal brain sections from an animal with a unilateral electrolytic lesion of the lateral hypothalamus in the region of the ascending catecholaminergic pathways. *d* shows the extent of the lesion, which resulted in ipsilateral depression of forebrain glucose uptake seen at the level of the caudatoputamen (*a*), globus pallidus (*b*), and thalamus (*c*). In *c*, the arrow denotes an area of increased glucose uptake at the rostral end of the lesion, probably representing a region of gliosis.

Glucose uptake was measured using a new technique developed by Sokoloff *et al.*, which facilitates determination of the rates of glucose uptake of individual structures within the central nervous system (CNS) by using tracer amounts of ^{14}C-2-deoxy-D-glucose (2DG)[3,4]. A pulse of the tracer is injected intravenously and 45 min allowed for completion of the phosphorylation of 2DG to ^{14}C-2-deoxy-D-glucose-6-phosphate by brain hexokinase. Autoradiographs, made from X-ray film exposed to cut sections of a brain from an injected animal, can be used to calculate the rates of glucose uptake of all the visualised brain structures. The autoradiographs can be used to compare quantitatively the relative rates of glucose uptake of such structures. Thus, the darker the particular area on an autoradiograph, the higher is the glucose uptake[3,4] in that area.

Recent studies using this technique have mapped qualitatively alterations of regional glucose uptake associated with alterations of functional activity. Regional, activity-related increases in glucose uptake occur in the mammalian CNS during sciatic nerve[4,5], vestibular[6], and olfactory[7] stimulation; during penicillin-induced seizures[4]; and during periods of swimming[8]. Decreases in glucose uptake follow unilateral eye enucleation and unilateral occlusion of an external auditory canal[4]. Such decreases resulted from elimination of well defined sensory inputs with limited projections. We wished to extend such studies to the metabolic alterations following destruction of intrinsic diffusely-projecting sys-

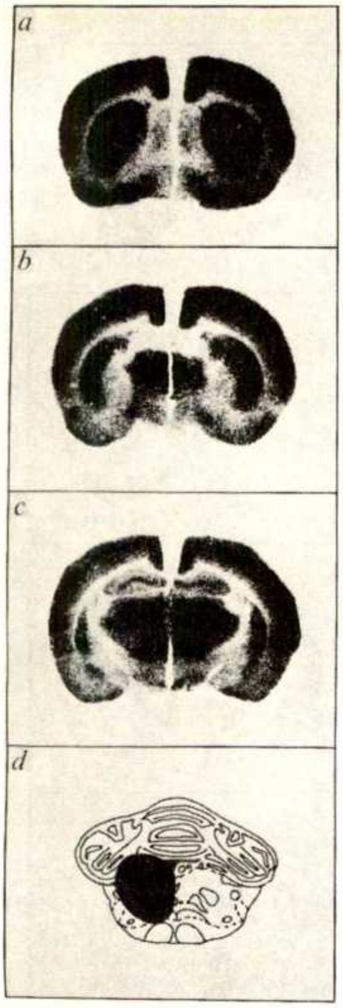


Fig. 2 Autoradiographs of coronal brain sections from an animal with a unilateral electrolytic lesion in the region of the locus coeruleus. *d* shows the extent of the lesion, which resulted in bilaterally symmetrical forebrain glucose uptake seen at the level of the caudatoputamen (*a*), globus pallidus (*b*), and thalamus (*c*).

tems. To do so, various lesions of the ascending catecholaminergic pathways were made.

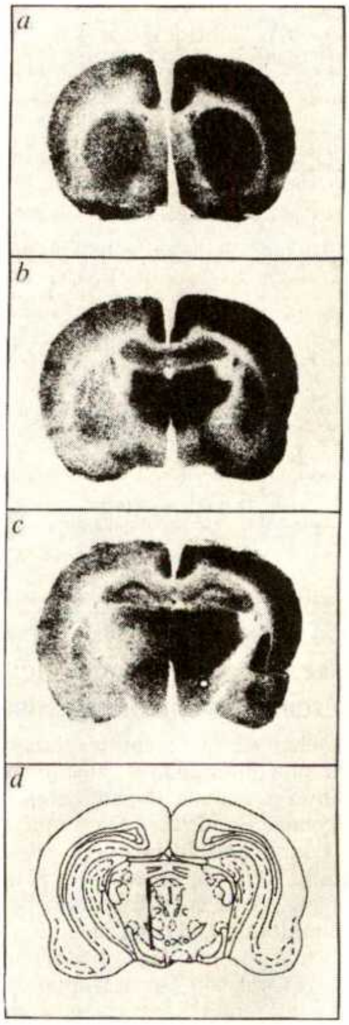
A total of 45 Sprague–Dawley albino rats, weighing approximately 150 g each, were anaesthetised with ethyl ether, and either electrolytic or 6-hydroxydopamine (6-OHDA) lesions were placed[8] stereotaxically in locations described below. For electrolytic lesions, 2 mA of current was passed for 30 s through stainless steel electrodes insulated to within 0.5 mm of their tips. 6-OHDA is believed to cause selective destruction of catecholamine-containing neurones[9]. For chemical lesions, 1 mg of 6-OHDA dihydrobromide (base) and 0.1 mg ascorbic acid were dissolved in 0.5 ml sterile saline and 4 μl of the solution (8 μg 6-OHDA) was injected over 4 min using a Hamilton syringe.

After lesioning, rats were housed in individual cages with food and water *ad libitum* for 72 h. They were then injected intravenously through the tail vein with 50 μCi ^{14}C-2DG (New England Nuclear, 53 Ci mol^{-1}) in 0.25 ml normal saline. After injection, animals were restrained in individual holders for 45 min and then killed with an overdose of sodium pentobarbital. Brains were removed quickly and frozen in isopentane cooled to −70 °C with solid CO$_2$. Frozen sections (30 μm) of brain were cut on a cryostat, dried quickly and autoradiographed on X-ray film as described previously[10].

We have made unilateral electrolytic lesions of the lateral hypothalamic area in the region of the ascending catecholaminergic pathways[2,11]. Nine animals were subjected to such a lesion and 8 had autoradiographs as shown in Fig. 1. The glucose uptake of structures rostral and ipsilateral to the lesion was depressed compared with that of the contralateral side; parts affected were wide ranging and included frontal cerebral cortex, caudatoputamen, parts of thalamus and other subcortical regions. The olfactory bulbs, hippocampus and structures caudal and ipsilateral to the lesion seemed unaffected. Control animals without lesions showed no such asymmetries.

To find out whether the ascending noradrenergic fibres cause this metabolic asymmetry, we made unilateral electrolytic lesions in the region of the locus coeruleus in four animals. These lesions were large (Fig. 2*d*), destroying much of the pons unilaterally at this level, so both the locus coeruleus and ascending noradrenergic fibres from more caudal cell groups (A1, A2, A5) were destroyed. In all these animals, forebrain glucose uptake was bilaterally symmetrical (Fig. 2). Similarly, brains of rats with unilateral ($n=4$) 6-OHDA lesions of locus coeruleus all had bilaterally-symmetrical forebrain glucose uptake. Their autoradiographs were similar to those in Fig. 2. Finally, unilateral electrolytic lesions 1–2 mm further anteriorly ($n=3$), destroying the A7 cell group and all the other

Fig. 3 Autoradiographs of coronal brain sections from an animal with a unilateral 6-OHDA lesion aimed at the substantia nigra. *d* shows the appearance of the cannula track; the lesion resulted in ipsilateral depression of forebrain glucose uptake seen at the level of the caudatoputamen (*a*), globus pallidus (*b*), and thalamus (*c*).



ascending noradrenergic fibres, likewise had symmetrical forebrain glucose uptake.

On the other hand, of 13 animals with unilateral 6-OHDA lesions aimed at the substantia nigra, 4 produced autoradiographs showing a metabolic asymmetry (Fig. 3) similar to that seen after unilateral lateral hypothalamic lesions (Fig. 1). Unilateral electrolytic lesions involving substantia nigra and ventral tegmentum resulted in depressed ipsilateral forebrain glucose uptake in 4 of 6 additional rats, and the autoradiographs were similar to those in Fig. 3.

Our lesions of noradrenergic fibres in midbrain and pons resulted in bilaterally-symmetrical autoradiographs; therefore the ipsilateral depression of forebrain glucose uptake seen after lateral hypothalamic lesions cannot be due to ipsilateral destruction of noradrenergic fibres alone. A forebrain metabolic asymmetry can be reproduced, however, by 6-OHDA lesions around the substantia nigra. Because 6-OHDA can destroy both dopaminergic and noradrenergic fibres[9], we cannot yet determine whether destruction of ascending dopaminergic fibres alone is sufficient to cause an ipsilateral depression of forebrain glucose uptake or whether simultaneous destruction of both dopaminergic and noradrenergic fibres is required. In either case, we do know that destruction of at least ascending dopaminergic fibres is required.

Our low success rate in animals with 6-OHDA lesions around the substantia nigra (4 of 13) is perhaps due to the relatively large volume occupied by the catecholaminergic elements at this level and so to the difficulty of destroying it all. At the level of the lateral hypothalamus, however, ascending catecholaminergic fibres are gathered together as bundles, and their complete interruption would seem more guaranteed. It is also possible that we destroyed other non-catecholaminergic fibres as they pass through the lateral hypothalamus, and their interruption may have contributed to the high success rate seen there.

The normal level of forebrain glucose uptake in the awake, restrained rat seems to depend, at least, on intact ascending dopaminergic pathways. We speculate that these fibres may modulate forebrain glucose uptake in the intact animal, but we do not know whether this modulation is first mediated through changes in cell firing rates or through the specific dopamine-sensitive adenylate cyclase[12,13]. Finally, perhaps some of the functions attributed to brain dopamine[14,15] are somehow related to its control of forebrain metabolic activity.

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Transport of globin mRNA from nucleus into cytoplasm in differentiating embryonic red blood cells

Erythropoiesis in chick embryos provides a useful experimental system to study specific gene regulation during embryonic differentiation. As they mature, red blood cells become almost totally committed to the synthesis of one set of gene products—haemoglobin peptide chains. Thus, the transcriptional and post-transcriptional processes involved in the expression of the globin genes can be conveniently studied in erythroid cells undergoing maturation.

It is the events that occur in erythroid cells at stages before and during the onset of haemoglobin synthesis that are of the most interest. Very little is known, however, about these early processes, one reason being the lack of an appropriate system whereby precursor erythroid cells at a sufficiently early stage—that is, before haemoglobin synthesis—can be obtained in adequate amounts and purity. This report describes experiments which delineate the initial pattern of transport of a eukaryotic messenger RNA (mRNA) (globin mRNA), from the nucleus into the cytoplasm during cellular differentiation. The results indicate that the synthesis, transport, degradation and translation of globin mRNA in embryonic erythroid cells seem to follow a rigorously regulated and coordinated pattern during embryonic differentiation. Globin mRNA is synthesised and retained exclusively in the nuclei during the initial 24 h of culture. This is followed by an almost complete transfer of globin mRNA from the nuclei into the cytoplasm within the next 24 h. Globin mRNA is thereafter predominantly in the cytoplasm. The relative amounts of globin mRNA in the nuclei and cytoplasm change reciprocally, although the total amount of cellular globin mRNA does not vary significantly during erythroid maturation. Haemoglobin becomes detectable only when 75% of the total cellular globin mRNA is present in the cytoplasm.

Erythroid cells at the erythroblast and later stages of maturation are present in the embryonic circulation of the chick, but the majority of these cells have already begun to synthesise haemoglobin[1]. To this end, we have established a culture system in which precursor chick erythroid cells can grow and undergo complete maturation *in vitro*. As a result of the culture conditions and the unique nature of the erythroid cell surface, clean and homogeneous populations of erythroid cells at various stages of maturation can easily be obtained[2].

In a previous report, we followed the initial synthesis of globin mRNA during maturation of cultured erythroid cells using a very sensitive and specific molecular probe for detecting and quantitating chick globin mRNA—^{3}H-labelled, single-stranded DNA complementary to embryonic chick globin mRNA (globin cDNA)[3]. The results indicated that significant amounts of globin mRNA were already present in the proerythroblasts by 24 h of culture. These results confirmed the observations obtained using actinomycin D as an inhibitor of RNA synthesis, which showed that haemoglobin synthesis in cultured erythroid cells was no longer sensitive to actinomycin D by 24 h of culture[2]. This separation in the time of the initial synthesis of globin mRNA and the first appearance of haemoglobin was also observed in intact embryos both by direct measurement of globin mRNA using globin cDNA[3] as well as indirectly, using actinomycin D (ref. 4).

The observations on the timing of the initial synthesis of globin mRNA during erythroid differentiation led to considerations of post-transcriptional regulation of the expression of globin genes. It is now generally accepted that globin mRNAs are very stable molecules[5–7]. Very little is known, however, about the events that occur from the

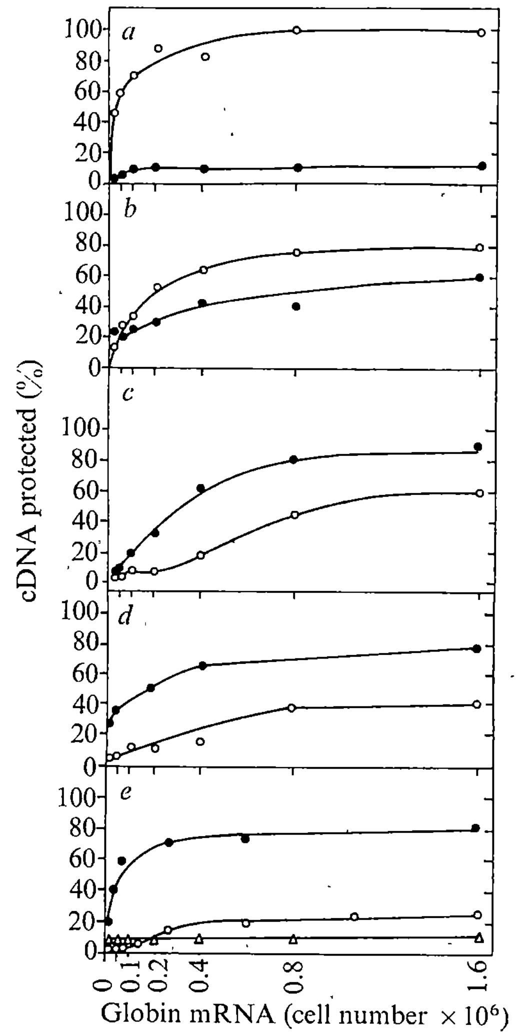


Fig. 1 Hybridisation of 7-d embryonic chick globin cDNA to nuclear and cytoplasmic RNA from erythroid cells at different times of culture. Erythroid cells were collected from the culture medium after various culture periods and washed several times with isotonic saline. Aliquots were taken for cell counts using a haemocytometer counting chamber and for preparation of slides. The slides were fixed in methanol, stained with benzidine reagent for haemoglobin and counterstained with mixed Wright Giemsa. The sensitivity of benzidine is estimated to be in the range of a few picograms haemoglobin per cell[11]. A total of 200 cells was scored with respect to their reaction to benzidine, and the percentage benzidine-positive cells calculated. The separation of nuclei from cytoplasm was accomplished as follows: the cell pellet was resuspended in 9 volumes buffer containing 0.01 M NaCl, 0.01 M Tris, pH 7.4, 0.003 M $MgCl_2$, 0.5% NP40 and 100 µg yeast transfer RNA (tRNA) for 2 min. Nuclei were pelleted by centrifugation at 1,400*g* for 15 min and the supernatant collected. The nuclei were washed once with the same buffer except no yeast tRNA was added. The supernatants were pooled, and 100 µg yeast tRNA was added to the nuclear pellet. RNA was extracted from the nuclear pellet and the supernatant using the phenol–chloroform–isoamyl alcohol technique. The ^{3}H-cDNA input was 300–350 TCA-precipitable c.p.m. per hybridisation reaction. The actual nanogram quantities of chick RNA used per reaction could not be determined because of the presence of yeast tRNA carrier, and therefore was normalised to the number of erythroid cells from which the RNA was extracted. Total cellular RNA extracted from cultured embryonic chick heart fibroblasts was used as control. ○, Nuclear RNA; ●, cytoplasmic RNA; △, total cellular RNA from embryonic chick heart fibroblasts. *a*, 24 h, 0% benzidine+; *b*, 30 h, 0% benzidine+; *c*, 44 h, 2% benzidine+; *d*, 50 h, 15% benzidine+; *e*, 72 h, 60% benzidine+.

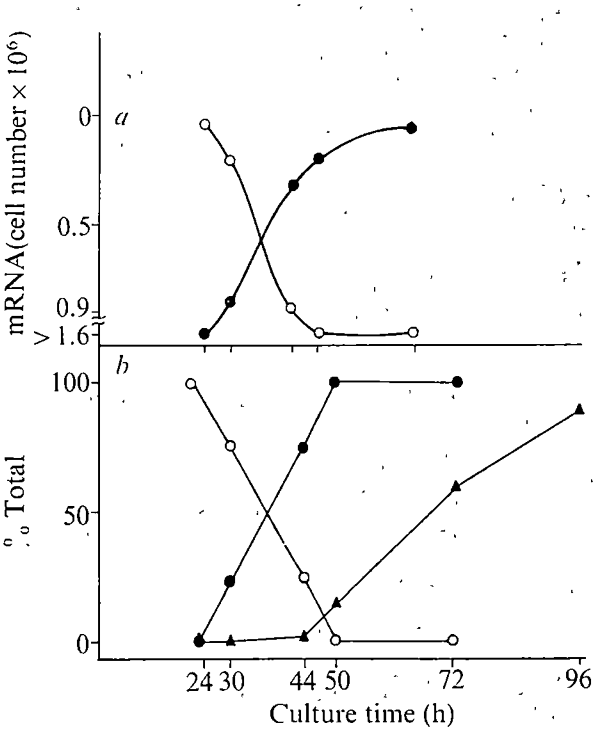
time of the initial synthesis of globin mRNA in the nuclei until the time when they are used for globin synthesis in the cytoplasm. It is reasonable to assume that the mRNA molecules must somehow move from the nuclei into the cytoplasm. But when and in what fashion mRNA transport occurs is hitherto unknown.

The experimental design used globin cDNA as a probe to quantify, as well as establish, the intracellular location of globin mRNA in erythroid cells during differentiation. Erythroid cells were collected after various times of culture, the nuclei were separated from the cytoplasm and the globin mRNA content of each fraction measured using labelled globin cDNA: cDNA was prepared using globin mRNA isolated from 7-d chick embryonic red cells as template[3]. Molecular hybridisation was carried out using the technique described by Ross *et al.*[8], except that 5-µl reaction mixtures were used. The amount of labelled cDNA protected by hybridisation with homologous RNA was determined by digestion with *Aspergillus oryzae* S1 nuclease as described previously[3].

Figure 1*a–e* shows the results of the hybridisation reactions between globin cDNA and RNAs extracted from the nuclei and cytoplasm of cells after various culture times. The percentage of cDNA protected was plotted against increasing amounts of RNA present in each reaction mixture. Total cellular RNA from cultured chick fibroblasts was used as control (Fig. 1*e*).

The results show that the initial transport of globin mRNA from nuclei into cytoplasm in differentiating erythroid cells follows a well regulated time course. Globin mRNA is synthesised and retained in the nuclei during the initial 24 h of culture, whereas very little, if any, is present in the cytoplasm. A considerable amount of globin mRNA

Fig. 2 *a*, Amounts of globin mRNA in nuclei and cytoplasm of erythroid cells at different times of culture. Ordinate shows amount of globin mRNA needed to give 50% protection of the cDNA in each reaction. Units were normalised to the number of erythroid cells from which the RNA was extracted. ○, Globin mRNA in nuclei; ●, globin mRNA in cytoplasm. *b*, Relative amounts of globin mRNA in nuclei and cytoplasm of erythroid cells and cells containing haemoglobin at different times of of culture. ○, Globin mRNA in nuclei; ●, globin mRNA in cytoplasm; ▲, cells containing haemoglobin.



then moves into the cytoplasm during the next 24 h of culture such that, by about 36 h, there are equal amounts of globin mRNA in the nuclei and the cytoplasm. By 44 h and thereafter, globin mRNA is predominantly in the cytoplasm.

From the 50% protection levels of cDNA in the hybridisation reactions, it is possible to estimate the absolute, as well as the relative, amounts of globin mRNA present in the two cellular compartments after different times of culture (Fig. 2*a* and *b*). The most striking observation is that the transport of globin mRNA does not follow immediately on synthesis. It is possible that this delay of transport may be due to the processing of mRNA from precursor molecules. The signal for the initiation of transport is unknown. Furthermore, since there is no overall change in the total amount of cellular globin mRNA during the 3-d culture period, this maximum level of globin mRNA is maintained regardless of its intracellular location. Note also that the amount of globin mRNA in the cytoplasm is a direct reciprocal of the amount of globin mRNA in the nuclei. There are several possible explanations to account for this observation: one is that globin mRNA is stable and does not turn over, so that once it is synthesised during the initial 24 h of culture, it remains intact in the cells for the duration of the culture period; a second possibility is that there is no new synthesis nor degradation of globin mRNA during the 24 h when it is being transported into the cytoplasm, but a steady state of synthesis and turnover is established thereafter. Neither of these possibilities seems likely, however, when we consider that the erythroid cells divide approximately once every 10–14 h at this time[8,9], but that no dilution of globin mRNA on a per cell basis occurs. A more likely explanation is that new globin mRNA is synthesised during this time, but the rates of synthesis, transport and degradation are coordinated such that the relative amounts of globin mRNA in the nuclei and cytoplasm change reciprocally, but the total amount of cellular globin mRNA remains unchanged. Evidence for this possibility awaits a thorough study of the rates of synthesis as well as rates and site(s) of degradation of globin mRNA in differentiating chick erythroid cells.

With regard to haemoglobin synthesis, the results show that haemoglobin becomes detectable only after 75% or more of total cellular globin mRNA is present in the cytoplasm (Fig. 2*b*). It is possible, however, that globin synthesis commences immediately after the first molecule of globin mRNA appears in the cytoplasm, but cannot be detected as haemoglobin until some time later. Studies are under way to establish the time when globin mRNA is first used for globin synthesis.

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Effect of ribothymidine in specific eukaryotic tRNAs on their efficiency in *in vitro* protein synthesis

RIBOTHYMIDINE (m^5U or rT), the most common methylated nucleoside found in tRNA, is always at the 23rd position from the 3′ end in the nucleotide sequence GTψCPurine[1,2]. So far ribothymidine has been found in essentially all prokaryotic tRNAs which function in protein synthesis, but not in several eukaryotic 'elongator' tRNAs[3–6]. In the case of wheat germ, all the glycine tRNAs, several species of threonine tRNA and one species of tyrosine tRNA have an unmodified uridine in the position usually occupied by ribothymidine (ref. 5 and K.B.M., D. Marcu, and B.S.D., manuscript in preparation). To determine the significance of the absence of rT in these tRNAs, we have methylated them enzymatically with a crude preparation of *E coli* rT-forming uracil methyltransferase and then compared their efficiency with that of the unmodified tRNAs in a wheat germ cell-free protein synthesising system directed by various natural mRNAs. We report here that these tRNAs function significantly more efficiently in protein synthesis when lacking rT and that the inhibitory effect of rT can be reversed by the polyamine spermine.

The existence of a class of tRNAs containing an unmodified uridine in place of rT is especially significant in view of the evidence suggesting that rT and the tetranucleotide in which it is located are involved directly in the role of a tRNA in protein biosynthesis[7–16]. Using a crude rT-forming uracil methyltransferase isolated from *E. coli* MRE 600 and *S*-adenosyl-L-methionine (SAM) as a methyl donor, we methylated *in vitro* the specific rT-lacking tRNAs from wheat germ. Analysis of the methylation reaction showed that rT was the only product, and in the case of pure wheat germ $\text{tRNA}_{\text{I}}^{\text{Gly}}$, all the rT was found at the 23rd position from the 3′ end, as is normal in other tRNAs (ref. 5). About 18% of crude wheat germ tRNAs lack rT and are substrates for methylation: this agrees with the level of rT detected in crude wheat germ tRNA by Littlefield and Dunn[17]. Crude wheat germ tRNA was methylated to a level of 0.06 mol of rT produced per mol of crude tRNA, which corresponds with about 30% of the available tRNA substrates. There was essentially no degradation of tRNA during methylation, as shown by the level of aminoacylation of glycine and leucine tRNAs (Table 1) and the absence of an observable change in the elution position of crude wheat germ tRNA and $\text{tRNA}_{\text{I}}^{\text{Gly}}$ on Sephadex G-100 before and after methylation (data not shown).

Initial experiments were performed with a wheat germ cell-free protein synthesising system[18] using tobacco mosaic virions (TMV) RNA, brome mosaic virions (BMV) RNA or chorion polysomal RNA obtained from the chorionating follicular cells of *Anthereae polyphemus* as mRNAs. Analysis of the protein products produced on sodium dodecyl sulphate (SDS)-polyacrylamide gels gave results similar or identical to those reported before for these mRNAs[19–22], and confirmed the fidelity of the translation described. But this wheat germ protein-synthesising system could not be used directly to study protein synthetic activity of specific *in vitro* methylated tRNAs because of its high content of endogenous tRNA. We therefore fractionated the wheat germ system and essentially removed the endogenous tRNAs as described before[23–24]. tRNA was then clearly needed for the incorporation of ^{3}H-leucine and ^{3}H-glycine in the translation of either TMV RNA, BMV RNA or chorion polysomal RNA (Table 2, lines 1 and 2 and Fig. 1*a*). The extent of protein synthesis with the sham-methylated or untreated crude tRNA controls described above was essentially indistinguishable for all mRNAs tested (Table 2, lines 3–6). The use of an equivalent amount of 6% methylated crude tRNA, however, led to a significant reduction in protein synthesis for all mRNAs tested (Table 2, lines 7 and 8). Incorporation of ^{3}H-leucine decreased by 43% and 36% for TMV and BMV RNAs, respectively, and the amount of ^{3}H-glycine incorporated with chorion mRNAs decreased by 54% compared with untreated or sham-methylated tRNA.

The time course of protein synthesis directed by TMV RNA was observed in the presence of untreated, sham or partially methylated wheat germ tRNAs. As Fig. 1*b* shows, protein synthesis during the first 60 min was inhibited with *in vitro* methylated tRNA compared with untreated or sham-methylated tRNA, and the degree of inhibition depended on the level of *in vitro* methylation.

To study specific tRNAs involved in the inhibitory effect, pure (1.5 nmol per absorbance unit) 100% methylated tRNA$_1^{\mathrm{Gly}}$ was mixed with untreated crude wheat germ tRNA and the mixture was tested for protein synthetic activity. With BMV RNA translation, no effect on ^{3}H-glycine incorporation was observed (Table 2, lines 9 and 10). There was, however, 48% inhibition of chorion mRNA translation (Table 2, lines 11 and 12). Thus, methylated tRNA$_1^{\mathrm{Gly}}$ is not involved in the inhibitory effect on BMV RNA translation but inhibits chorion mRNA translation. This was not surprising in view of the very high glycine content of the chorion proteins produced[22]: it was the reason we chose this mRNA for study.

Protein synthesis studies were then performed with precharged tRNA$_1^{\mathrm{Gly}}$ to establish that inhibition is at the level of the ribosome. No appreciable inhibition was observed for

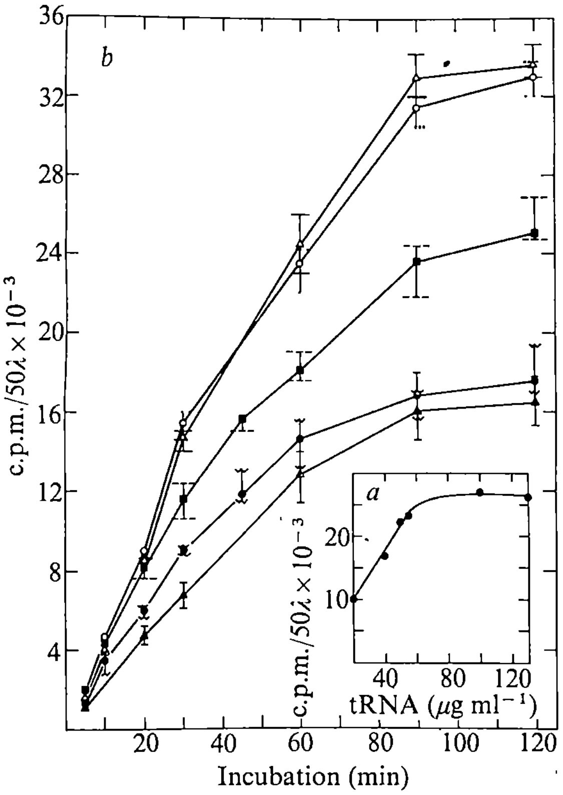

Fig. 1 *a*, Optimum concentration of crude wheat germ tRNA for the fractionated protein synthesising system. The levels of ^{3}H-leucine (2 Ci mmol^{-1}) incorporated into protein in a 50-λ protein synthesis assay (with washed ribosomes and an $(NH_4)_2SO_4$-concentrated DEAE-cellulose-treated S100) directed by 4 μg of TMV RNA is shown at various exogenously supplied levels of crude wheat germ tRNA. Reactions were terminated by adding 1 ml of 5% trichloroacetic acid (TCA), heated to 90 °C for 15 min, then cooled and collected on to Whatman GF/A filters which were dried at 150 °C and counted in Omnifluor–toluene as described before[18]. Each point represents the average of at least three determinations with a fluctuation not greater than 5%. *b*, Kinetics of protein synthesis directed by TMV RNA with *in vitro* methylated, sham-methylated and untreated crude wheat germ tRNA. All protein synthesis assays were performed with 2.5 μg of the particular tRNA in 50 λ. Samples of 5 λ were taken at the times indicated, precipitated, counted as before[18], and corrected for the total incorporation in 50 λ. All five assays were performed in triplicate except for the experiments with non-preincubated tRNA and 6% methylated tRNA, which were performed in duplicate. The 60-min time point for the non-preincubated tRNA sample was obtained in six other experiments with a deviation of not more than 10%. The 60-min time point for the 6% methylated tRNA assay was obtained in three other determinations, also with a fluctuation of not more than 10%. In addition, the 60-min time points for the preincubated, 1.2% and 3.2% methylated tRNAs were reproduced in at least two other assays. The same level of inhibition of methylated tRNA compared with sham or untreated tRNA controls observed at 2.5 μg of tRNA per 50-λ reaction were also observed at a tRNA concentration of 5.0 μg per 50-λ reaction. Proteins were labelled with ^{3}H-leucine (2 Ci mmol^{-1}). ○, Untreated; △, sham-methylated; ■, 1.2% methylated; ●, 3.2% methylated; ▲, 6% methylated.

Fig. 2 *a*, Protein synthetic activity directed by TMV RNA in the fractionated cell-free system with crude tRNA (50 μg ml^{-1}) at various concentrations of spermine. The reactions were carried out as described in Fig. 1 and ref. 18. Each point was obtained at least in triplicate and the fluctuation was not greater than 12%. *b*, Kinetics of protein synthesis directed by TMV RNA with *in vitro* methylated and sham-methylated crude wheat germ tRNA in the presence of spermine. All protein synthesis assays were performed with 2.5 μg of the particular tRNA sample in 50 λ. Samples of 5 λ were taken at the times indicated, precipitated and counted as before[18], and corrected for the total incorporation in 50 λ. The sham-methylated and 3.2% methylated tRNAs were determined in triplicate and the 1.2% and 6% methylated tRNAs were obtained in duplicate. The 60-min time points of all four assays were redetermined in two separate experiments. Proteins were labelled with ^{3}H-leucine (2 Ci mmol^{-1}). Symbols as in Fig. 1.

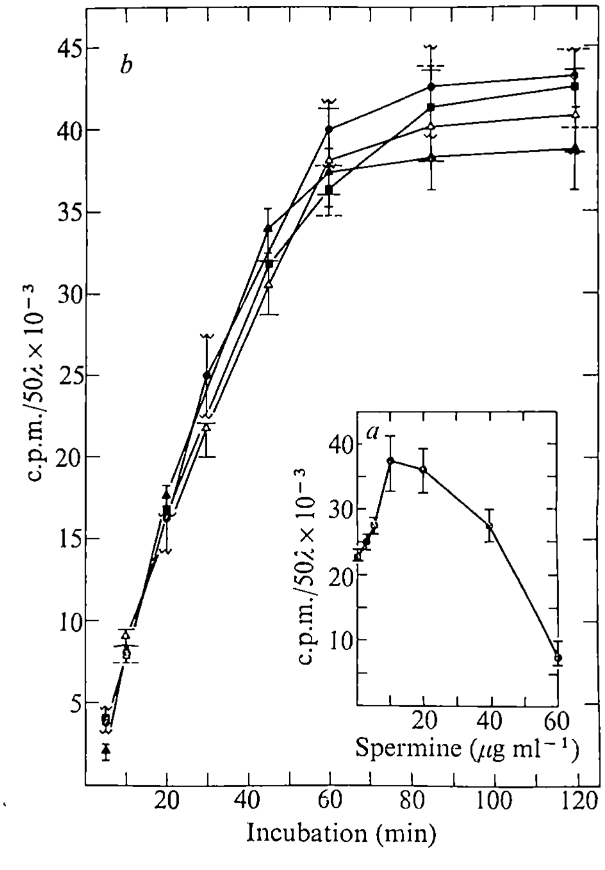

Table 1 Aminoacylation of *in vitro* methylated, sham-methylated and untreated crude wheat germ tRNA

tRNA	^{3}H-leucine incorporation (c.p.m.)	^{3}H-glycine incorporation (c.p.m.)
Untreated	2,450	1,040
Sham-methylated	2,400	1,100
1.2% Methylated	2,260	940
3.2% Methylated	2,250	915
6% Methylated	2,200	975

Performed in a final volume of 50 λ containing 50 mM Tris buffer, *p*H 7.6, 0.5 mM EDTA, 20 m $\mathrm{MMgCl_2}$, 2.5 mM ATP (disodium salt), 10 μM amino acid (^{3}H-leucine, 2 Ci mmol^{-1} or ^{3}H-glycine, 1 Ci mmol^{-1}), 2.5 μg of each tRNA sample and 10 λ of ribosome and tRNA-free wheat germ protein extract. The reaction was incubated at 30 °C for 30 min. Values listed are c.p.m. above a tRNA control performed in duplicate with ~ 10% deviation, and, in the case of methylated tRNAs, they are also corrected for the radioactive methyl label. Sham-methylation refers to the methylation reaction performed in the absence of SAM.

BMV RNA translation (Table 2, lines 13 and 14), which agreed with the comparison of uncharged *in vitro* methylated $\mathrm{tRNA_1^{Gly}}$ and uncharged, untreated $\mathrm{tRNA_1^{Gly}}$ (Table 2, lines 9 and 10). Chorion mRNA-directed protein synthesis was inhibited using precharged, methylated $\mathrm{tRNA_1^{Gly}}$ ~48% (Table 2, lines 13 and 14). The degree of inhibition is essentially identical to that observed without precharging the tRNA before protein synthesis (Table 2, lines 11–13), showing that the inhibition does not occur at aminoacylation.

Polyamines stimulate natural mRNA translation in various cell-free systems[18,25–28]. When an optimum concentration of spermine (10 μg ml^{-1}) was added to the cell-free system (Fig. 2*a*), the inhibitory effects of methylated $\mathrm{tRNA_1^{Gly}}$ on chorion mRNA translation and of methylated bulk tRNA on TMV RNA translation were eliminated (Table 2, lines 15 and 16 and Fig. 2*b*). The relative protein synthetic activity was increased to the same extent for both sham-methylated and untreated tRNA controls, but the increase was much greater for the methylated tRNA.

The ability of spermine to abolish the inhibitory effect of methylated tRNA may reflect a specific effect of spermine on tRNAs and their function in protein synthesis, perhaps mediated by altering the conformation of the tRNAs. Polyamines bind to ribosomes[29] and tRNAs (refs 30–33), and spermine differentially stimulates the translation of specific proteins directed by adenovirus mRNAs so as to reflect the relative levels of the specific proteins made *in vivo*[28].

The inhibitory effect on cell-free protein synthesis resulting from *in vitro* rT formation in specific wheat germ tRNAs may explain the absence of rT in these tRNAs *in vivo*. These same tRNAs also lack rT in 14-d-old wheat seedlings (data not shown). rT formation with these specific tRNAs may result in a less active tRNA conformation which is further altered in the presence of spermine to improve tRNA efficiency. The level of spermine varies widely in different organisms[29], and the level of spermine in wheat germ is not known and may be insufficient to prevent the inhibitory effects observed. The level of spermine (and perhaps even rT) could vary and constitute an effective regulatory mechanism for protein biosynthesis.

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We thank Drs Barbara Filner for TMV-infected leaves;

Table 2 Protein synthetic "activities" of *in vitro* methylated, sham-methylated and untreated tRNAs in a tRNA-dependent cell-free system directed by natural mRNAs

tRNA	^{3}H-amino acid	Spermine (10 μg ml^{-1})	TMV inc.* (c.p.m.)	BMV inc.† (c.p.m.)	Chorion inc.‡ (c.p.m.)
(1) None	Gly§	—	—	190	100
(2) None	Leu§	—	200	170	—
(3) Untreated	Leu§	—	22,500	13,500	—
(4) Untreated	Gly§	—	—	1,478	2,570
(5) Sham-methylated	Gly§	—	—	—	2,477
(6) Sham-methylated	Leu§	—	23,000	12,920	—
(7) 6% Methylated crude tRNA	Leu§	—	13,000	8,250	—
(8) 6% Methylated crude tRNA	Gly§	—	—	—	1,150
(9) Untreated $\mathrm{tRNA_1^{Gly}}$‖	Gly§	—	—	1,396	—
(10) Methylated $\mathrm{tRNA_1^{Gly}}$‖	Gly§	—	—	1,470	—
(11) Untreated $\mathrm{tRNA_1^{Gly}}$‖	Gly¶	—	—	—	15,830
(12) Methylated $\mathrm{tRNA_1^{Gly}}$‖	Gly¶	—	—	—	8,230
(13) ^{3}H-Gly-$\mathrm{tRNA_1^{Gly}}$**	—	—	—	780	3,020
(14) ^{3}H-Gly-(methyl) $\mathrm{tRNA_1^{Gly}}$**	—	—	—	600	1,580
(15) Untreated $\mathrm{tRNA_1^{Gly}}$‖	Gly¶	+	—	—	30,750
(16) Methylated $\mathrm{tRNA_1^{Gly}}$‖	Gly¶	+	—	—	33,000

Hot TCA-precipitable radioactivity above background (−mRNA control) for 50-λ reactions incubated for 60 min at 30 °C as before[18]. All assays contained 2.5 μg of tRNA unless otherwise noted. Data was obtained at least in duplicate and in some cases in quadruplicate or sextuplicate (TMV data) with an average deviation of 10%. The maximum protein synthetic activity of the fractionated cell-free wheat germ system directed by TMV RNA was approximately 1/8 the level (with and without spermine) observed for the unfractionated extract. This drop in activity was also noted for BMV RNA (1/5) and for chorion polysomal RNA (1/7). The high level of activity of the crude extract was only consistently obtained if the extract was prepared quickly (total preparation time 75–90 min) and stored in liquid N_2 (up to several months) or at −80 °C (up to several weeks). Probably the time required to prepare the fractionated system (10–12 h) resulted in significant loss of protein synthetic activity. This drop in activity was also observed for the crude unfractionated extract after several weeks of storage at −20 °C.

* 4 μg TMV RNA per 50-λ assay.

† 4 μg BMV RNA per 50-λ assay.

‡ 10 μg crude polysomal RNA per 50-λ assay.

§ ^{3}H-glycine, 1 Ci mmol^{-1}; ^{3}H-leucine, 2 Ci mmol^{-1}.

¶ ^{3}H-glycine, 20 Ci mmol^{-1}.

‖ 20% of the 2.5 μg of crude tRNA added was $\mathrm{tRNA_1^{Gly}}$ or methylated $\mathrm{tRNA_1^{Gly}}$.

** 2.5 μg of either methylated or untreated $\mathrm{tRNA_1^{Gly}}$ precharged with ^{3}H-glycine (20 Ci mmol^{-1}) + 2.5 μg of crude untreated tRNA. A standard 50-λ protein synthesis reaction was performed with a 50-fold excess of glycine to prevent deacylation and reacylation of ^{3}H-glycine. Reactions were performed in duplicate with ~ 10% deviation.

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Myoglobin of the orangutan as a phylogenetic enigma

THE primary structure of the myoglobin of nine anthropoid primates is known from previous work on three ceboids, two cercopithecoids and four hominoids[1,2]. The most striking result of these investigations has been the nature of residue 110. With the exception of the cercopithecoids and hominoids this position is occupied by alanine in all mammalian myoglobins known, including those of the South American monkeys. In the Old World monkeys, however, residue 110 is serine, and in man and the three apes so far investigated it is a cysteine. It is noteworthy that two of the codons for Ala can change to codons for Ser by a single point mutation, and each of these can change to a codon for Cys by a further single point mutation. There seemed, therefore, to be an evolutionary sequence Ala → Ser → Cys at this position in myoglobin[3] In the α chain of human haemoglobin and that of many other animals the homologous position is also occupied by cysteine[4]. The myoglobin residue at position 23 is also of particular interest among the primates, being serine in the gibbon and the monkeys investigated so far, whereas it is glycine in all other mammalian myoglobins for which the primary structure is known, including those of man, chimpanzee and gorilla.

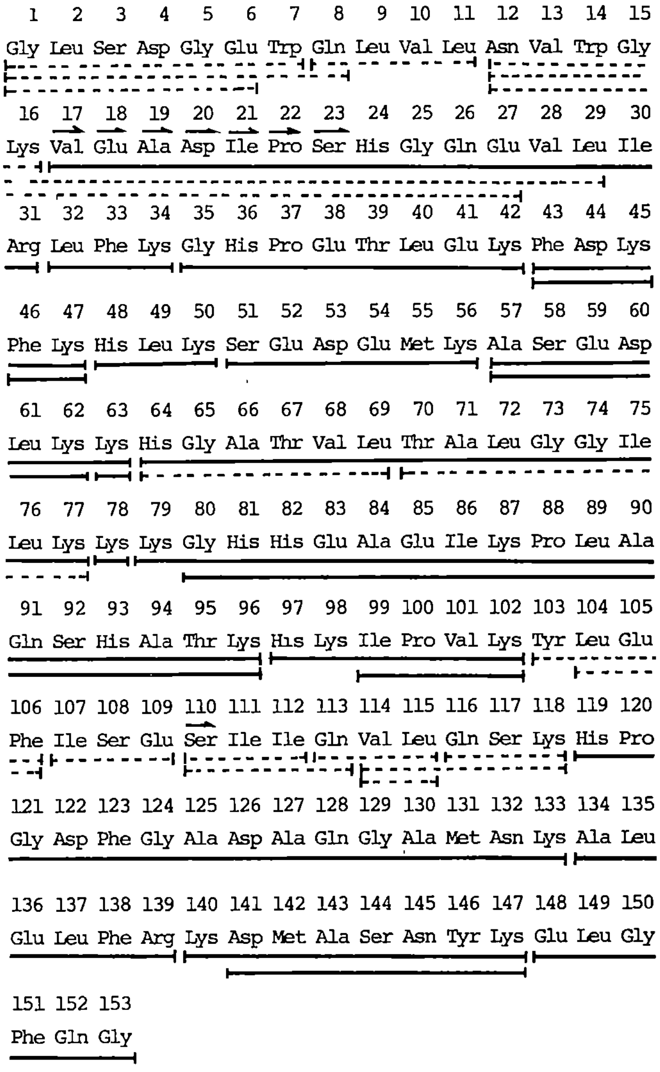
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Gly Leu Ser Asp Gly Glu Trp Gln Leu Val Leu Asn Val Trp Gly
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Lys Val Glu Ala Asp Ile Pro Ser His Gly Gln Glu Val Leu Ile
31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Arg Leu Phe Lys Gly His Pro Glu Thr Leu Glu Lys Phe Asp Lys
46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
Phe Lys His Leu Lys Ser Glu Asp Glu Met Lys Ala Ser Glu Asp
61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
Leu Lys Lys His Gly Ala Thr Val Leu Thr Ala Leu Gly Gly Ile
76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
Leu Lys Lys Lys Gly His His Glu Ala Glu Ile Lys Pro Leu Ala
91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
Gln Ser His Ala Thr Lys His Lys Ile Pro Val Lys Tyr Leu Glu
106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
Phe Ile Ser Glu Ser Ile Ile Gln Val Leu Gln Ser Lys His Pro
121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
Gly Asp Phe Gly Ala Asp Ala Gln Gly Ala Met Asn Lys Ala Leu
136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
Glu Leu Phe Arg Lys Asp Met Ala Ser Asn Tyr Lys Glu Leu Gly
151 152 153
Phe Gln Gly

Fig. 1 Orangutan (*Pongo pygmaeus pygmaeus*) myoglobin. Comparison with human myoglobin shows only two differences at positions 23 and 110 where the human residues are Gly and Cys, respectively. Methods used have been described elsewhere[13]. ——, Tryptic peptides; – – – –, peptic peptides; ⇀, dansyl-Edman degradation.

The amino acid sequence of the myoglobin of the orangutan (Fig. 1) differs from that of man at only two positions, surprisingly 110 and 23. At position 110, orangutan resembles the Old World monkeys by having serine, whereas the other hominoids have cysteine. At position 23 the orangutan resembles the gibbon and monkeys by having serine, rather than glycine as in man, chimpanzee and gorilla. Bearing in mind recent discussion of the phylogeny of catarrhine primates[5] these findings were unexpected and we have sought to investigate their possible implication.

If the conventional classification of the hominoids is assumed to reflect their phylogenetic relationships, then

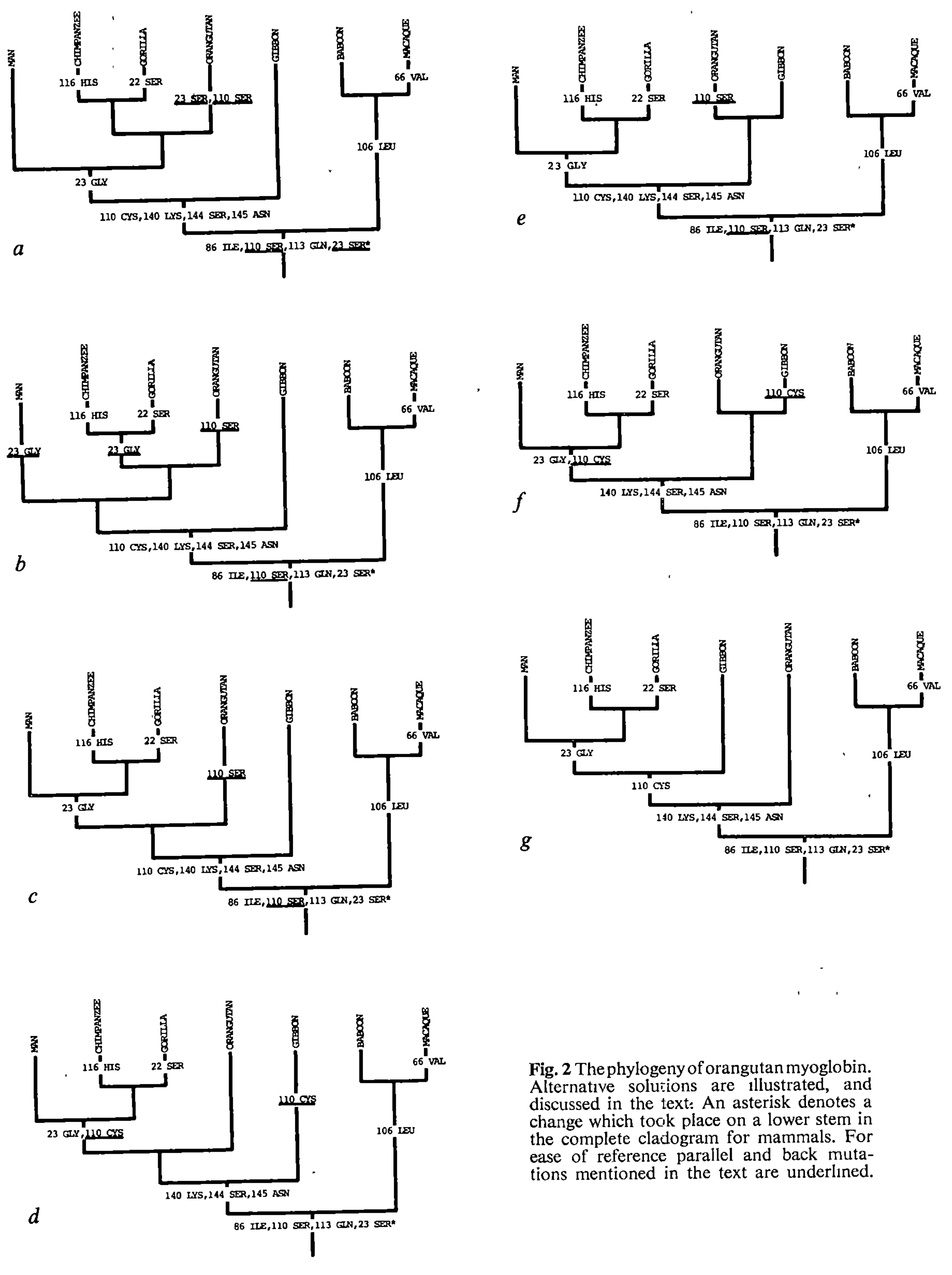

Fig. 2 The phylogeny of orangutan myoglobin. Alternative solutions are illustrated, and discussed in the text. An asterisk denotes a change which took place on a lower stem in the complete cladogram for mammals. For ease of reference parallel and back mutations mentioned in the text are underlined.

the orangutan would share immediate common ancestry with the chimpanzee and gorilla, all being included within the Pongidae, whereas the Hominidae would form a separate lineage leading to man. This pattern of phylogeny is shown in Fig *2a* and *b* In both of these cladograms, to account for the observed pattern of residues at the two positions 23 and 110 within the phylogeny of the anthropoids, a total of six single point mutations (hits) is required. In cladogram *2a*, 23 Ser and 110 Ser are both shown as back mutations in the line leading to the orangutan, whereas in cladogram *2b* the mutations to 23 Gly are shown as parallel events in the lineage leading to man and in the common stem leading to chimpanzee and gorilla, but 110 Ser remains as a back mutation in the orangutan lineage.

On the other hand, it is generally believed that the family Hominidae originated within the family Pongidae and so it is possible that the orangutan lineage branched from the pongid stem before the divergence of the Hominidae. In this event the chimpanzee and gorilla would share common ancestry with man subsequent to the divergence of the orangutan. This pattern, shown in cladograms *2c* and *d*, can be resolved more economically than *2a* and *b*.

It requires only 5 hits to account for the observed distribution of residues at positions 23 and 110. In cladogram 2*c*, 110 Ser remains as a back mutation in the orangutan lineage whereas in cladogram 2*d* 110 Cys is shown as being the product of parallel mutations in the gibbon and in the common stem leading to man, chimpanzee and gorilla.

An equally economical pair of solutions, involving exactly the same series of mutations as those in cladograms 2*c* and *d*, can be obtained by linking the orangutan to the gibbon (rather than to man, chimpanzee and gorilla) as shown in cladograms 2*e* and *f*. Such a phylogenetic pattern might be taken to imply that there is a fundamental division between African and Asian apes, and that the lineages leading to orangutan and to gibbon separated in Asia rather than in Africa. This is not necessarily so because any hypothesis relating African and Asian apes demands the presence of a viable migration route, and so the divergence between the orangutan and gibbon lineages might have occurred in Africa, along the migration route, or in Asia.

If one seeks the most economical solution to the phylogeny of the residues at positions 23 and 110 then one has to construct cladogram 2*g* (4 hits), in which the gibbon is so placed that it shares 110 Cys with man, chimpanzee and gorilla, and 23 Ser with the orangutan. This pattern implies that the line leading to the orangutan separated from the hominoid stem before that leading to the gibbon, which is contrary to the current consensus of opinion. Nevertheless, in trying to assess the relative merit of the alternative phylogenetic patterns discussed above, we have taken note of recent statements which seem to indicate that opinion on primate phylogeny is still in a state of flux.

Several years ago, Goodman[6] and Sarich[7] cast serious doubts on the phylogenetic pattern represented by cladograms 2*a* and *b*. The weight of immunological evidence and the sequences of fibrinopeptides[8], support patterns 2*c* and *d*. Nevertheless, Groves[9] expressed the view that gibbons were probably descended from the *Dryopithecus* complex, and he regarded their specialisations as being associated with the development of brachiation and reduction in size. Such a view could be accommodated by cladograms 2*e* and *f*, and Schultz[10], having reviewed skeletal features within the Hylobatidae, concluded that the siamangs resemble the great apes more closely than do other gibbons, and that this resemblance was particularly to the orangutan. On the other hand, Andrews and Groves[11] have little doubt that the gibbons branched off first from the hominoid common stem and their suggestion that *Dryopithecus africanus* is representative of a stage of hominoid evolution when *Pongo* had already branched off the stem but *Homo* and *Pan* had not yet separated, brings us back to cladograms 2*c* and *d*.

The only evidence known to us which provides support for the most economical phylogenetic pattern for myoglobin (2*g*) is given by Darga *et al.*[12], who reported that "with rabbit anti-human albumin serum, gorilla and chimpanzee seem to be almost identical to man while gibbon and siamang show slight divergence and orangutan shows yet more divergence although only about a third as much as Old World monkeys". On the other hand, for rabbit anti-human transferrin, gibbon and siamang diverge more than orangutan, and Darga *et al.*[12] concluded that the weight of immunological and molecular evidence indicated that within the Hominoidea, from the lineage leading eventually to man and the living African apes, an early branch led to the Hylobatidae and a later one to the orangutan.

If we accept the phylogenetic pattern of cladograms 2*c* and *d*, then we are forced to accept either a back mutation or a parallel mutation to arrive at the myoglobins of living hominoids. The same number of hits is required for cladograms 2*e* and *f* the pattern of which implies that orangutan and gibbon are phylogenetically equidistant from man. This pattern provides the least demanding circumstances for resolving the conflicting evidence from albumin and transferrin. If we reject cladogram 2*g* then we are rejecting the most economical solution. The evidence of other molecules is awaited with interest.

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Host DNA replication or excision repair requirement for ultraviolet induction of bacteriophage λ lysogens

THE mechanism of prophage induction by radiation or chemical agents is unknown, although during the past decade a variety of hypotheses have been advanced[1–6]. Sussman and Ben Zeev[7] have presented biochemical data that seem to favour the suggestion that DNA intermediates in repair of DNA damage are competitors with the prophage operators for repressor binding. When sufficient repressor is bound to these regions none remains for prophage repression and so induction follows[7]. A consequence of this hypothesis is the prediction that induction

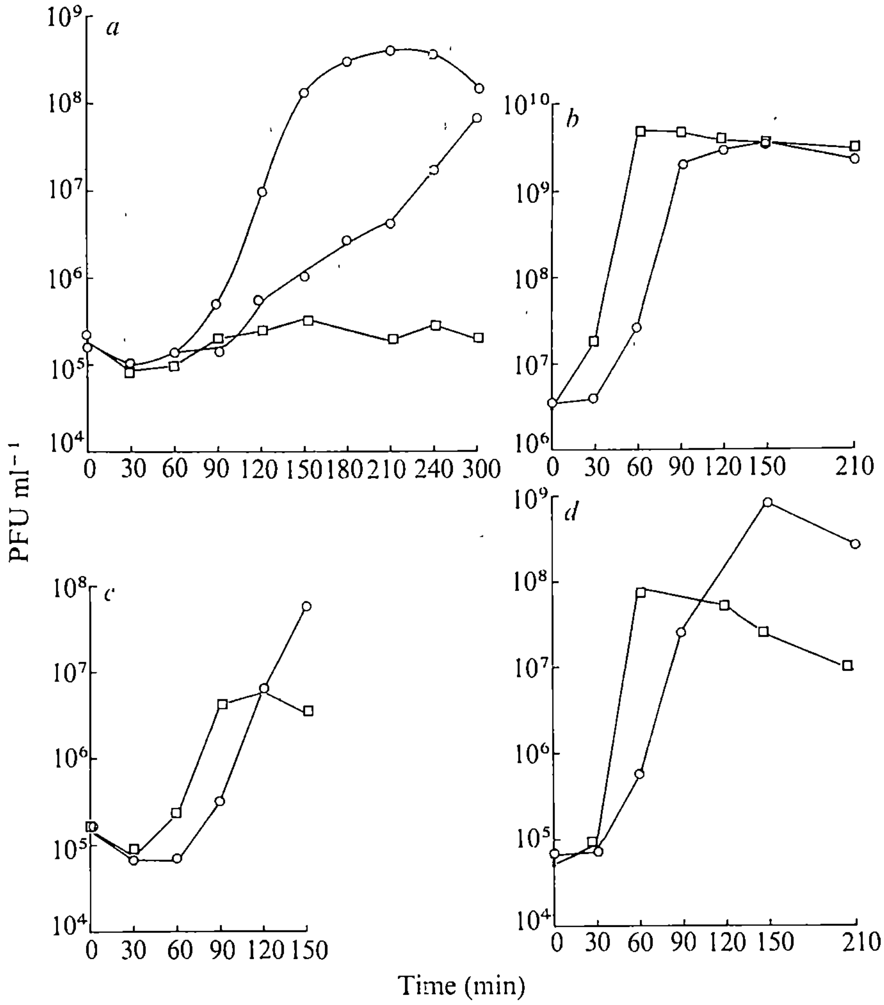

Fig. 1 *a*, *b* and *c*, Cells were grown to about 10^8 cells ml^{-1} in 10 ml 1% tryptone (Difco), 1% NaCl, 0.2% maltose and 0.01% yeast extract at 34 °C. Cultures were shifted to 42 °C for 1 h and poured into a pre-warmed 600-ml beaker in a 42 °C water bath. At $t = 0$ the culture was irradiated with 254-nm light. All subsequent steps were carried out in subdued light A sample was then transferred to a flask at 34 °C, and the remaining culture was incubated at 42 °C. At intervals samples were removed, shaken with chloroform and titred for plaque-forming units (PFU) on C600 (*uvr*$^+$ *rec*$^+$) by standard techniques. *a*, A second sample was removed from the 42 °C culture at $t = 60$ min and incubated at 34 °C (middle curve). *d*, Cells were grown at 34 °C until $t = 0$, when they were irradiated with the rest of the experimental protocol as in *a*, *b* and *c*. ○, Incubated at 34 °C; □ incubated at 42 °C. *a*, *dnaA uvrB* λ lysogen irradiated with an incident ultraviolet dose of about 55 J M^{-2}, yielding a cell survival of 0.02%. This dose was found to yield maximum phage induction in control experiments. Cell density at $t = 0$: 9 × 10^7 ml^{-1}. *b*, *dnaA* λ lysogen. Incident dose 110 J M^{-2}; cell survival, 3%; cell density at $t = 0$, 1.3 × 10^8 ml^{-1}. *c*, *uvrB* λ lysogen. Incident dose 55 J M^{-2}; cell survival, 0.09%; cell density at $t = 0$, 1.6 × 10^8 ml^{-1}. *d*, *dnaA uvrB* λ lysogen without 42 °C preincubation. Incident dose 55 J M^{-2}; cell survival, 0.08%; cell density at $t = 0$, 1.8 × 10^8 ml^{-1}.

should not occur in the absence of these normal repair processes. Here we test and verify this prediction.

Only two repair processes are known to alter the structure of DNA during repair· excision repair, which is absent in *uvrA*, *uvrB* and *uvrC* mutants[8], and post-replication repair, which seems to be a consequence of the passage of the DNA replication point past a damaged region of DNA[9,10].

Both forms of repair were eliminated using a bacteriophage λ lysogen of a *dnaAuvrB* double mutant. The *dnaA* mutation of Abe and Tomizawa[11] is temperature sensitive in the initiation of DNA replication, and grows normally at 34 °C. When shifted to 42 °C replication already started continues until complete but no new rounds of replication are initiated. DNA synthesis was undetectable in lysogens of these mutants after 1 h at 42 °C as measured by the incorporation of H^3-thymidine. DNA synthesis resumed shortly after the lysogens were returned to 34 °C. The *uvrB5* used here was obtained from strain AB2499 of Howard-Flanders[8].

Lyosgens were grown at 34 °C to a density of about 10^8 cells ml^{-1} and shifted to 42 °C for 1 h They were then irradiated at 42 °C and a sample transferred to 34 °C with the remainder kept at 42 °C. At intervals, samples were removed and assayed for free plaque-forming units.

Figure 1*a* shows that induction does not occur when the irradiated culture is held at 42 °C, whereas it does in the aliquot transferred to 34 °C. When the transfer was delayed until 1 h after irradiation, induction was also delayed (Fig. 1*a*, middle curve). Figure 1*b*, *c* and *d* shows control experiments, in *b* and *c* the same experimental protocol was followed with lysogens of single *dnaA* or single *uvrB* mutant hosts In both cases induction occurs in cultures held at 42 °C as well as at 34 °C, indicating that either excision or replication alone is adequate for induction. In Fig. 1*d* the *dnaAuvrB* lysogen was irradiated before the shift to 42 °C. Induction occurs at 42 °C, an indication that there is no defect in bacteriophage maturation in this double mutant at 42 °C.

Experiments with the radiomimetic antibiotic mitomycin C at 1 μg ml^{-1} as the inducing treatment have yielded quantitatively similar results.

As is shown in Fig. 2, little induction was observed over a wide range of ultraviolet doses in non-replicating, non-excising lysogens. The slight inducibility at 5 J m^{-2} has been observed repeatedly and may indicate either residual replication or excision repair, or the presence of alternate, minor pathways of prophage induction.

The absence of ultraviolet induction in non-replicating, non-excising lysogens found here is in disagreement with earlier results of Monk and Gross, who found induction in similar conditions[12]. The Monk and Gross experiments differed from these principally in the technical detail of dilution after irradiation. When this dilution step was used with the *Escherichia coli* lysogens of the present study, induction was obtained in cultures held at 42 °C. It is thus possible that dilution somehow initiates replication in a small fraction of the cells, a possibility which was not examined by Monk and Gross.

Although the results reported here can be taken to favour the alternate binding site hypothesis of Sussman and Ben Zeev, they do not rule out the possibility that the

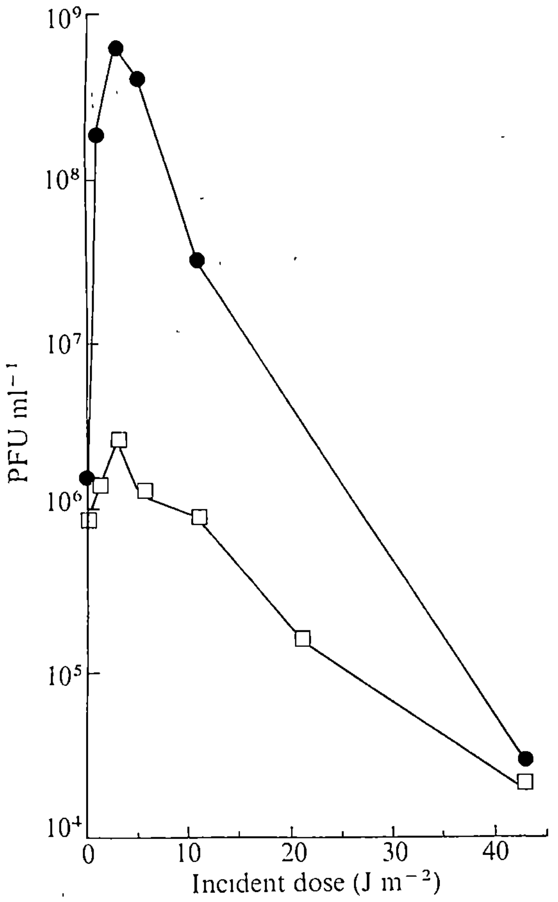

Fig. 2 Dose response of ultraviolet induction: 10 ml *dnaAuvrB* mutant was grown to a concentration of about 2×10^8 cells ml^{-1} at 34 °C in tryptone salt medium described in Fig. 1, collected by centrifugation and resuspended in 20 ml M9 medium[13] supplemented with 0.5% decolourised casamino acids, 0.01% thiamine and 0.2% glucose This culture was incubated with shaking at 42 °C for 1 h, and irradiated as described in Fig. 1. At various doses two samples of 1 ml each were removed with a preheated pipette. One sample was incubated at 42 °C, the other at 34 °C for 3 h. Great care was taken to prevent cooling of the samples before the 3-h incubation was over Chloroform (0.1 ml) was added to each of the incubation tubes, and the tubes shaken at 34 °C for 2 min. The titre of free phage was assayed as before. ●, Incubated at 34 °C. □, incubated at 42 °C.

repair intermediates are also alternative binding sites for repressors of other operons. For example, it is possible that the resulting derepression could induce an 'error-prone' repair system which in turn would cause bacteriophage induction[6] In view of Sussman's[7] observations, however, that the λ repressor binds to DNA isolated from ultraviolet-irradiated and mitomycin-treated cells[7], competition between prophage operators and repair intermediates for λ repressor seems to be the simplest way to account for the observed results.

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Structure of insulin in 4-zinc insulin

DURING his experiments on slow acting clinical preparations of insulin, Schlichtkrull found that a second form of rhombohedral insulin crystals appeared when the sodium chloride ion concentration in the solution was 6% or more[1]. The new crystals contained four zinc ions per hexamer of insulin compared with two in the original crystals. Both forms crystallised in the space group R3 and had similar lattice constants: $a=82.5$ Å, $c=34.0$ Å for 2-zinc insulin, and $a=80.7$ Å, $c=37.6$ Å for 4-zinc insulin crystals[2] Both, from an early application of the rotation function[3], also seemed to be very similar in structure, and to contain in each asymmetric unit, two insulin molecules related to one another by non-crystallographic twofold axes of symmetry. We have now found, from electron density calculations on 4-zinc pig insulin, that the apparent similarity of the crystal structures conceals a surprisingly large change in the conformation of one only of the two insulin molecules in the unit.

The electron density map of 4-zinc pig insulin was phased on data from three isomorphous derivatives; a lead acetate derivative (3 sites) to 2.8-Å resolution; a mercuric acetate-2-hydroxybenzaldehyde derivative (3 sites) to 4.5 Å; and an iodo derivative formed by covalent attachment of iodine to tyrosine (4 sites: one of which proved to be a change in off-axial zinc ion occupancy or temperature factor) to 4.5 Å (ref. 4); measurements on anomalous scattering were made on each crystal. The contributions of the last two derivatives in the phasing calculations were small and the overall figure of merit 0.67. The map is easily interpretable, however, by comparison with that of 2-zinc insulin[5]; over one of the pair of insulin molecules in each unit the electron density is superimposable, over the other marked changes in residue arrangement can be followed. Only a few residues, which are on the protein surface, are not well defined.

In 4-zinc insulin, as in 2-zinc insulin, there are three equivalent dimers associated around the threefold axis to form a hexamer; the two structures are compared in Fig. 1 There are two zinc ions on the threefold axis in 2-zinc insulin, attached through B10 histidine residues to the two very similar molecules, I and II, of each insulin dimer. In 4-zinc insulin, one zinc ion appears on the threefold axis, attached to molecules nearly identical with molecule II of 2-zinc insulin The remaining zinc ions occupy general sites surrounding the threefold axis, linked by much-changed molecules at position I (Fig. 2). The change is effected by a major rearrangement of the first eight residues of the B chain. These, originally extended, now follow a generally helical course continuing at an angle of about 30° in the direction of the main B9–B19 α-helix.

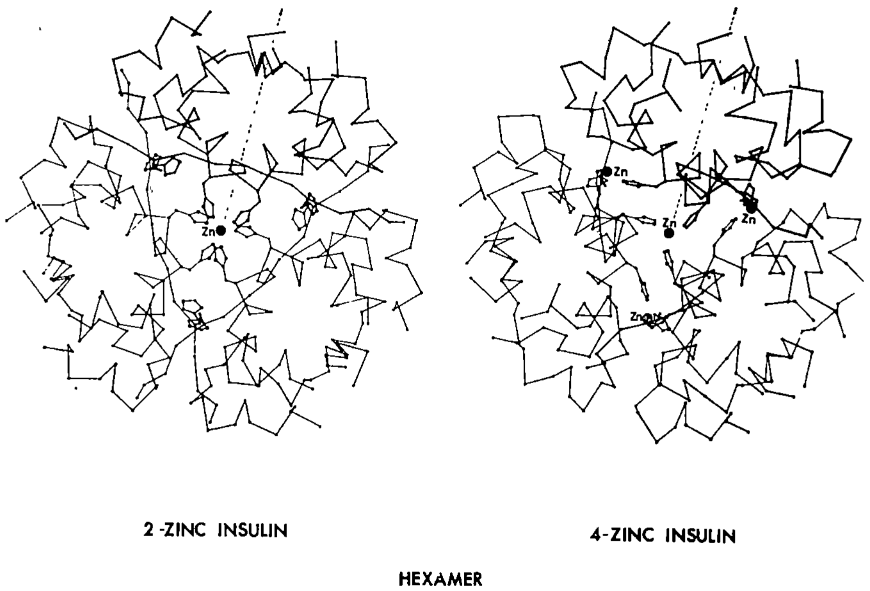

Fig. 1 A comparison of the 2-zinc insulin and 4-zinc insulin hexamers viewed down the threefold axis. Only α-carbon atoms, histidine side chains and zinc ions are shown. The local twofold axis of the dimers drawn in bold lines is also indicated.

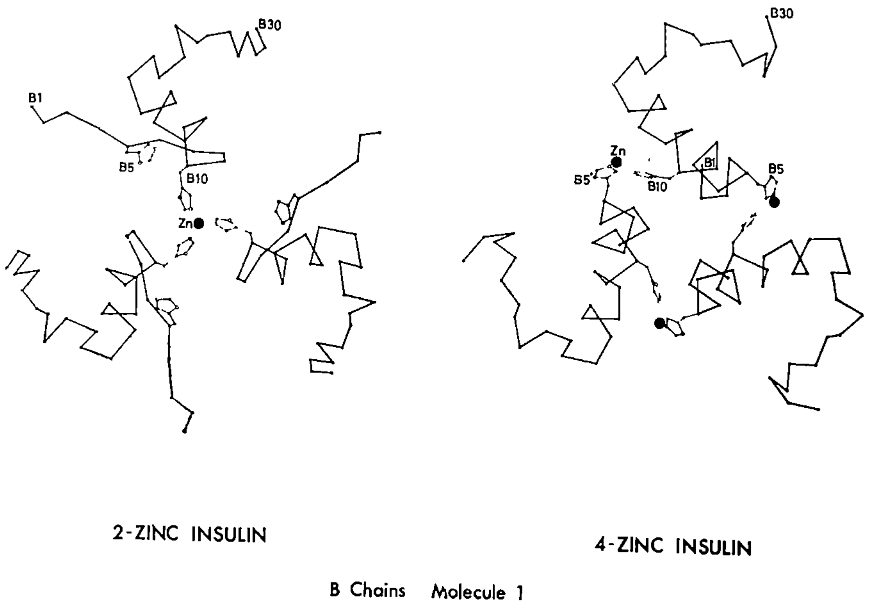

Fig. 2 An identical view to Fig. 1 comparing the molecule I B chains of 2-zinc insulin and 4-zinc insulin, illustrating the changed conformation and zinc coordination.

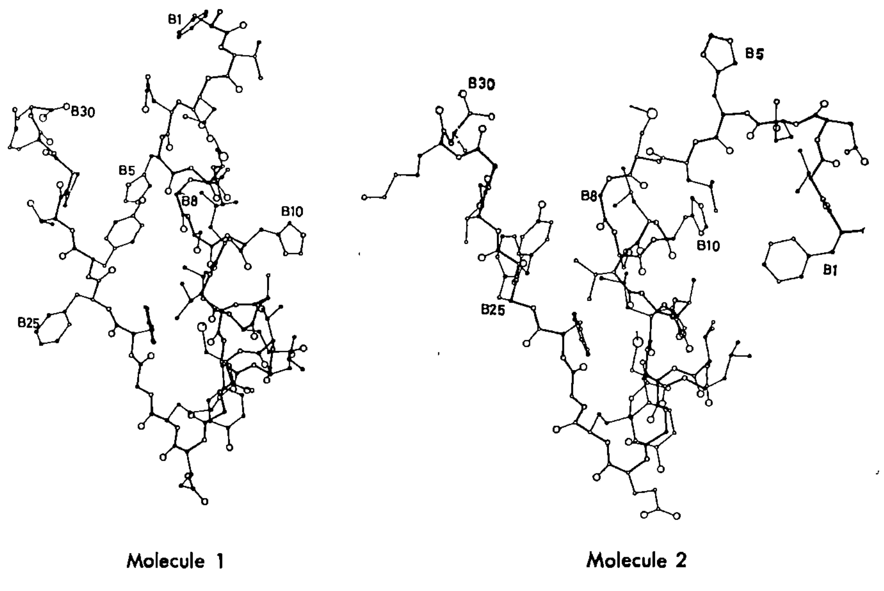

Fig. 3 The B chains of molecules I and II viewed from an equivalent direction.

The B5 histidine is moved in the transformations—15 Å across the cell, close to the B10 residue of a neighbouring molecule, which turns towards it. The zinc ion is held between these two histidine residues, tetrahedral co-ordination about it being completed by two water molecules. The change in the B-chain structure pivots about the B8 glycine residue which moves from the 'right-handed' amino acid conformation still present in molecule II, to the helical region of the Ramachandran plot.

The local twofold symmetry which is obeyed quite well in the 2-zinc insulin structure is therefore completely violated by the arrangement of the first eight B-chain residues of insulin molecules in 4-zinc insulin. Between B9 and B30, on the other hand (Fig. 3), the conformations of the two B chains are very similar. Correspondingly, the β-pleated sheet structure and the close non-polar contacts between them are unchanged in this region. The constancy of the structure here confirms our view that these B-chain contacts define the region of aggregation within the insulin dimer present in solution. The A chains of the two molecules in the 4-zinc insulin dimer are found to be quite similar to one another in conformation, but not in position relative to the B chains. The A chain of molecule I has moved in response to the movement of the A7–B7 disulphide link away from its usual contacts with the B-chain residues. As a consequence, a cleft appears within molecule I (compare Fig. 4). This encourages the asymmetric iodination in the crystal of the tyrosine side chains. The main reaction, which does not occur in 2-zinc insulin, is at tyrosine A19 (two sites with 36% and 26% substitution) and tyrosine B26 (one site with 19% substitution) of molecule I. The enhanced reactivity of molecule I in the insulin dimer is similar to the behaviour of enzyme complexes, such as malate dehydrogenase, in which only half the subunits are active[6].

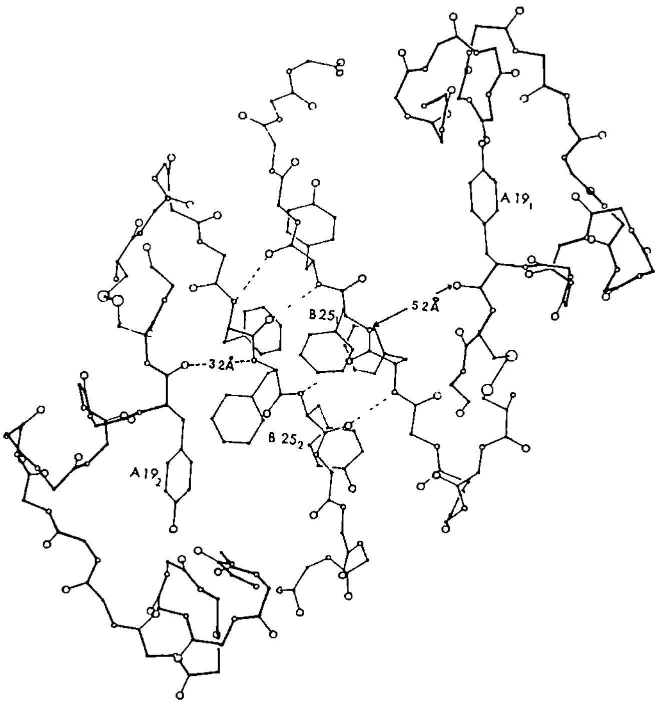

Fig. 4 Part of the 4-zinc insulin dimer viewed along the local twofold axis, showing the appearance of the cleft in molecule I. The region B1–B19 is omitted in both monomers and only the side chains of A19, A20, B19, B24, B25 and B26 are drawn.

The principal change we have observed, the coiling of the initial residues of the B chain, may be encouraged by the sequence in pig and cattle insulin, which is helix-forming in character. Preliminary experiments suggest that ions other than chloride may initiate the transformation, and that the ions enter the crystals. With sodium iodide the crystals formed are similar to, but not exactly isomorphous with, those obtained in sodium chloride solutions; difference electron density maps indicate that the ions enter at different positions on or near the threefold axis. We also have evidence that the 2-zinc crystals themselves can be transformed to 4-zinc insulin crystals simply by adding additional chloride to the mother liquor surrounding them, and that the transformation is reversible (G.A.B., G.G.D. and A. Lewitova, unpublished). The changes are shown by changed lattice constants and by the intensities of diffraction spectra in projection. As might be expected, variable crystal disorder occurs with crystal change; the pathway through the water between the molecules along which the peptide chains must move is clearly tortuous and difficult to follow.

Although the experimental conditions used to effect the transformation to 4-zinc insulin are far from physiological, the phenomena observed are very suggestive in relation to insulin action. 4-zinc cattle insulin displays markedly prolonged hypoglycaemic action and this has led to its use in clinical medicine. Since slow action is less pronounced in 4-zinc pig insulin, it seems that the change must be correlated mainly with different contacts between the molecules which affect solubility and thus insulin release. It is noticeable that the A10 residue, which varies between these species, makes a direct contact with the B5 histidine in the extended helix. The variation of the structure of insulin molecule I, and particularly the opening of the cleft between the A and B chains in the neighbourhood of tyrosine A19, does not seem to impair the overall biological activity of the insulin; evidently it is easily reversible. Many lines of evidence have implicated this region in the action of insulin. Our observations suggest that its flexible character may be one of the important factors in generating biological responses[7,8].

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Possible dosimeter for ultraviolet radiation

WHILE evaluating the weathering characteristics of the plastics polysulphone and polyphenylene oxide (PPO) we found that they both darkened when exposed to ultraviolet radiation[1]. We realised the potential of these polymers as monitors for ultraviolet radiation and are developing them for this use. PPO is now being used to monitor continuously solar ultraviolet radiation at forty sites throughout the world[2]. We are developing polysulphone as a possible personnel dosimeter to measure exposure of the skin to ultraviolet radiation. Two requirements for any ultraviolet dosimeter are: (1) that the wavelength response of the dosimeter should be similar to the erythemal action spectrum of human skin, and (2) that the dosimeter should have a monotonic response as the ultraviolet dose increases. The results presented here indicate that polysulphone might well satisfy these criteria.

The polysulphone used (P 1700 Union Carbide) has the structure shown in Fig 1. It has good stability to thermal[3], thermal oxidative[4] and high energy radiation[5]. It readily undergoes changes in surface properties when exposed outdoors. These changes are accentuated if the material is used as a film, and so this was the form we used

A high pressure xenon arc lamp was used in conjunction with a single grating monochromator to investigate the response of the polysulphone film to ultraviolet radiation. Irradiance was measured by a thermopile.

When exposed to ultraviolet radiation, polysulphone film was degraded and lost tensile properties At the same time the initial broad absorption band in the near ultraviolet extended with dose towards the visible. As a measure of the degree of degradation of the polymer, the change in absorbance measured at 330 nm (ΔA_{330}) was noted. This can be done on any standard ultraviolet spectrometer and takes about 10 s.

The change in absorbance was investigated as a function of incident ultraviolet dose for wavelengths ranging from 254 nm to 335 nm. (The film does not respond to wavelengths greater than 340 nm.) At each wavelength, measurements were made for increasing doses until the ΔA_{330} of the film reached approximately 0.3. The values of A_{330} of the unirradiated film were in the range 0.155 to 0.175. Figure 2 shows the results obtained for irradiation at 297 ± 5 nm, that is, at a bandwidth at half-maximum intensity of 10 nm.

For none of the wavelengths studied did the ΔA_{330} increase linearly with dose. The first differential of ΔA_{330} with respect to ultraviolet dose decreased as the dose increased. If, however, the dose–response curves for all wavelengths were normalised at an absorbance of 0.2 to eliminate the different wavelength sensitivities, the mean deviation of each curve from the 297-nm curve was less than 5% at all absorbances up to 0.2. The response of a film exposed to stable sunlight also showed this degree of agreement with the 297-nm curve up to an absorbance of 0.2. At higher absorbances, the agreement lessened; the shorter the wavelength, the less rapid was the increase in absorbance with increase in ultraviolet dose.

This nonlinearity in the response of the film to ultraviolet radiation was not due to oxygen starvation, for a film exposed under high vacuum showed a dose response almost identical to that of one exposed in air, and indicates the photolytic rather than the photo-oxidative nature of the process. The decrease observed in the response of the film at high doses was attributed to the protective filtering effect of the new absorption centres produced. This was supported by the observation that doping unexposed polysulphone film with a known ultraviolet absorbant, 2-hydroxybenzophenone, produced, on exposure to ultraviolet radiation, the same reduction in rate observed in an exposed but undoped sample with the same ΔA_{330}. Investigations with diphenyl sulphone revealed similar radiation sensitivity and build up of filtering products, which suggest that the diphenyl sulphone group in the polymer is responsible for the properties observed.

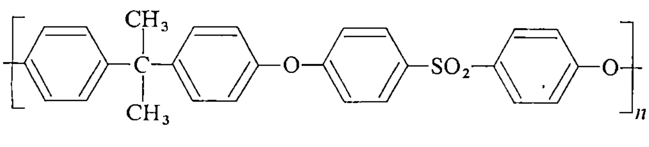


Fig. 1 The structural unit of polysulphone. To prepare the film, the polysulphone was first purified by reprecipitation: the chloroform solution of the polymer was added to a stirred volume of methanol and then the filtered polymer was dried in a vacuum oven at 60 °C. The film was made by spreading a 10% chloroform solution of reprecipitated polymer on a flat glass plate with an adjustable casting blade, producing a film between 36 and 44 μm thick. The film was removed from the glass plate, dried overnight in a vacuum oven at 60 °C and stored in the dark before use. The choice of film thickness was a compromise between minimising the absorption of wavelengths greater than 330 nm and achieving mechanical strength to facilitate handling. The film was mounted in a standard 5 × 5 cm photographic transparency holder, constituting the film badge. The badge was usually worn in a matt black holder to eliminate ultraviolet radiation reflected back from the subject.

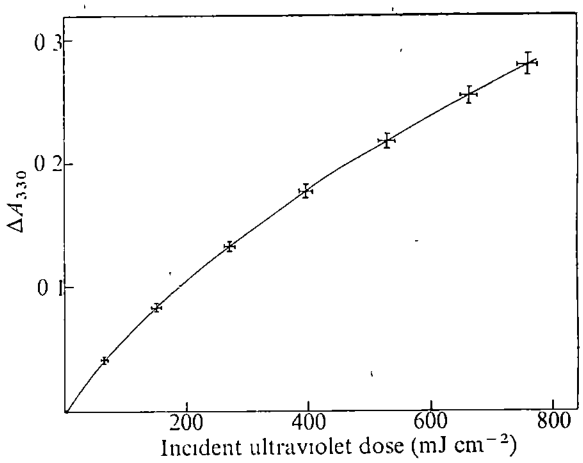


Fig. 2 The variation of ΔA_{330} with incident ultraviolet dose at 297 nm.

When stored, a previously exposed polysulphone film underwent a 'dark reaction', that is, the film exhibited a slight increase in absorbance. After 24 h the ΔA_{330} had increased by about 4%, after 1 week by about 5%. Allowance was made for this factor when necessary.

To derive the wavelength response of the film, the reciprocal of the dose required to produce a ΔA_{330} of 0.1 at each wavelength was plotted as a function of wavelength (Fig. 3). The results were normalised to unity at the most sensitive wavelength. In Fig. 3 the erythemal action spectrum of human skin[6,7] is compared with the action spectrum of polysulphone. Although the agreement is not ideal, it is possible to allow for this, if the relative spectral distribution of the incident radiation is known.

Samples of polysulphone film were irradiated at five levels of irradiance ranging from 0.056 mW cm^{-2} to 0.337 mW cm^{-2} to give equal doses of 250, 500, 750 and 1000 mJ cm^{-2} at a wavelength of 305 ± 5 nm. The resultant values of ΔA_{330} at each dose from the five intensities were within the experimental error of 5% of each other, which indicates that the response of the polysulphone film was independent of dose rate.

For wavelengths less than 315 nm, practically all the radiation is absorbed in a 40-μm film and so a change in the angle of incidence of the radiation will produce negligible changes in response. The refractive index of polysulphone between 315 and 330 nm was 1.63 and at an angle of incidence of 80° the increase in path length was about 25% relative to radiation normally incident on the film. At this angle of incidence, about 20% of the incident radiation was reflected, so the overall effect on the increase in absorbance was minimal.

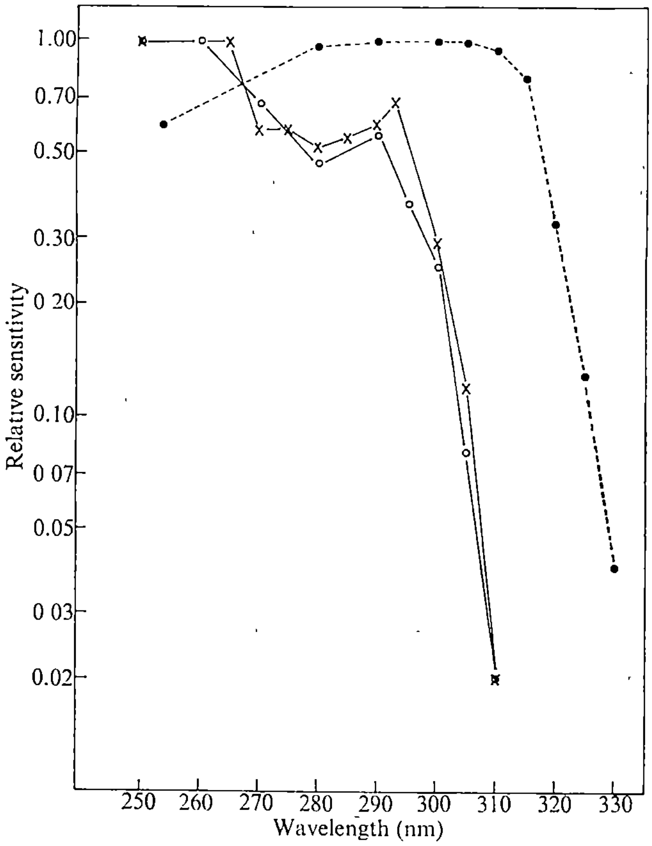
From a calibration curve of the type shown in Fig. 2 it is possible to relate the change in absorbance of a film irradiated by heterogeneous ultraviolet radiation of known relative spectral distribution to an equivalent exposure (F) of 297-nm radiation. The effective erythemal dose (D) at 297 nm is then given by

$$D = F\left[\frac{\int I(\lambda)E(\lambda)\mathrm{d}\lambda}{\int I(\lambda)G(\lambda)\mathrm{d}\lambda}\right] \qquad (1)$$

where $I(\lambda)$ is the relative spectral distribution of the incident radiation at wavelength λ, $E(\lambda)$ is the erythemal effectiveness at wavelength λ relative to that at 297 nm, assuming the mechanism of response does not change with wavelength and $G(\lambda)$ is the spectral sensitivity of polysulphone in terms of absorbance change, ΔA_{330}, at wavelength λ relative to that at 297 nm, as the biological effects produced by this wavelength have been studied extensively.

The results of this preliminary study of polysulphone as an ultraviolet dosimeter look promising. The method is being used to measure the ultraviolet exposure of bedridden geriatric patients, laboratory personnel working indoors for an 8-h day and hospital gardeners, with a view to estimating the amount of solar radiation needed for the synthesis of vitamin D in the skin. It is hoped that the results will help to quantify previous observations[8,9].

Fig. 3 Action spectra of polysulphone and human skin. ●, Polysulphone; ——, human skin; ×, ref.6; ○, ref.7.



Although the reproducibility of response of the polysulphone film to a known spectral distribution is of the order of 5%, it is important to know the uncertainty associated with the transformation of polysulphone response to an effective biological dose. This error depends on the nature of the ultraviolet environment and the action spectrum of the biological process under consideration. Careful attention must be paid to the action spectrum of the process to ensure that it declines to zero while the action spectrum of polysulphone is finite. The possibility of making the film thinner and therefore obtaining a better match with the erythema action spectrum is being investigated.

We thank Professor I A. Magnus for interest and constructive criticisms. B.L.D. thanks Mr J. R. Corfield for encouragement.

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Erratum

In the article "Similarities in the helical sequences of the repressor-binding sites in the *lac* and λ operators" by M. C. O'Neill (*Nature*, **260**, 550, 1976) the recurring face sequence displayed on page 552 should be

$$\begin{array}{ccc} \binom{T}{A} & \binom{A}{T} & \binom{C}{G} \\ \mathrm{AA} - - & \mathrm{CTC} - - & \mathrm{CG} \\ \mathrm{TT} - - & \mathrm{GAG} - - & \mathrm{GC} \end{array}$$

Correction

In the article "Nalidixic acid and bacterial chromosome replication" by G. C. Crumplin and J. T. Smith (*Nature*, **260**, 643; 1976) Figs 2 and 3 have been transposed.

Nature Index and Binders

The **Index** for 1975 is now available, price £2.25. Copies of the 1974 index are still on sale, price £3.00 **Binders** for the journal are also available at £7.00 for four (a year of *Nature* fits into four binders).

Postage is included in the above prices. Orders should be sent, accompanied by remittance to Macmillan Journals Ltd, Brunel Road, Basingstoke, Hampshire, England.

reviews

Slaves to the machine

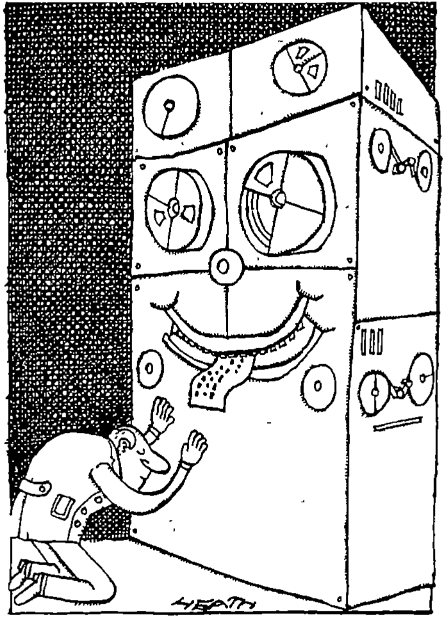
P. N. Johnson-Laird

Computer Power and Human Reason: From Judgment to Calculation. By Joseph Weizenbaum. Pp. xii+300. (Freeman: San Fransico and Reading, January 1976.) $9 95; £5 95

No computer centre is complete without one—a gaunt-faced hollow-eyed youth slaving over a hot teletype, working for hours on end long into the night and rarely pausing to satisfy his bodily needs. Should the time available to him on the machine begin to run out, his intensity of work rises to a feverish pitch: he lives dangerously, staking all on some irretrievable alteration of his program. Once off the machine, his life assumes a dull grey quality and is sustained only by sheaves of computer print-outs. He cannot wait to return to where the action is, and will sacrifice all social relations and obligations just to get back on to the system.

Who are these strange dedicated obsessive individuals? They are the new breed of slaves to the machine, the computer 'hackers'. And what are they doing? Almost invariably, they are developing some grandiose general all-purpose super-duper system that once completed will enable other less able programmers to write their own super-duper systems. Weizenbaum, in the most brilliant chapter of his provocative book, has delineated the psychology of computer hackers with a deep and convincing clinical expertise. He shows how the compulsive character of their work relates to a worldview similar to that of their second cousins, the gambling addicts. The computer provides the hacker with a universe, subtle but not malicious, over which he can exercise an absolute and god-like control. The programmer who is corrupted by this power is likely to develop great expertise, but he pays a price. His general all-purpose system is almost always an empty exercise in disembodied programming—that is to say, it fails to make intellectual contact with any other discipline.

The computer hacker is the central metaphor of Weizenbaum's book. He stands for the person who places a single-minded reliance on science as a source of understanding of life, who believes that man is nothing but a machine, and who cheerfully hands over responsibility for making decisions to computers. Weizenbaum's humanistic scruples are particularly offended by the equation of human thinking with computational processes, and therefore by the new scientific discipline of Artificial Intelligence (AI).



The aim of AI is to construct intelligent machines, and its practitioners are notably devoted to composing computer programs that can perceive the world, or analyse two-dimensional representations of it, and that can conduct conversations in natural language. The first programs capable of rudimentary versions of such skills were written some years ago, but it soon became obvious that it was extremely difficult to test them against human performance A cynic might say that it was for this reason that the goal of simulation was abandoned in favour of a science of the artificial. Weizenbaum, however, is no cynic; he advances a rather different argument against AI. Since some of his work laid the foundations for the new science, the impact of his book is comparable to that of an anti-vivisectionist tract written by a physiologist whose reputation rests on his operating skills.

What, in essence, is Weizenbaum's case? One can distill three main points from his book. First, certain tasks are too important to be handed over to a computer. It would be immoral as well as dangerous to make computers responsible for certain forms of psychotherapy. Such a proposal, relying on a program in fact devised by Weizenbaum, seems to have been the critical event that led to the present book. Second, artificial intelligencers suffer from hubris as an occupational disease. They are much given to promising to solve the riddle of the universe . . . tomorrow. "The only reason we have not yet succeeded in simulating every aspect of the real world is that we have been lacking a sufficiently powerful logical calculus. I am working on that problem." So spoke Professor John McCarthy in a perfect specimen of the megalomania that can grip the best of minds at the end of a long day's programming into the night. Weizenbaum, of course, considers that there are certain human propensities, such as the ability to feel desperate or to fall in love, that computers will never be able to display simply because they are not human beings. Third, AI programs are increasingly in danger of exceeding their progenitors' ability to understand them It is confidently expected that scene analysis programs will soon exceed 1,000K in size, and no-one is likely to be able fully to understand or to communicate their mode of operation.

These are serious criticisms of AI, but even with the worst will in the world they hardly convict the discipline of triviality or immorality. Current computers cannot pick their noses, nor can they fall in love. There is every reason to suppose that they could be devised to carry out the former task, and no reason other than a humanist conscience for supposing that they could not be devised to do the latter. It *is* a frightening thought—almost as frightening as the idea that our ancestors were apes and that we live on an insignificant planet revolving round an insignificant star. Weizenbaum deserves our gratitude for writing an instructive book that can be read with profit by anyone from a humanist to a computer scientist. It should force us to think more carefully about what we set our computers to do. It should force the artificial intelligencers to reappraise their discipline. No serious practitioner is likely thereby to abandon his science. And the hackers, of course, know that life is nothing but an enormous program devised by the Great Programmer in the sky. ☐

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Rudiments of physics

Elementary Particles and Symmetries (Documents on Modern Physics.) By Lewis Ryder. Pp. xxvi+249. (Gordon and Breach : New York, London and Paris, December 1975.) £9.80; £5.00 (International Student Edition).

LEWIS RYDER'S book grew out of lectures given to third-year undergraduates at the University of Kent, and it shows. In my opinion, it is not enough simply to tell students about particle physics. I share what I take to be the author's view that it is preferable, or at least equally important, to teach them how to calculate something—almost anything will do! Any like-minded teacher will find a wealth of material in this admirable book. Of course the topic of symmetries is well suited to this task The discrete symmetries (time reversal, charge conjugation, parity reversal), the additive conservation laws (charge, strangeness) and the non-abelian groups (isospin, SU_3) are all covered and applied to concrete examples of the strong, weak and electromagnetic interactions. Ten of the thirteen chapters are essentially free of phrases like: "It can be shown that . . .", which usually herald the onset of the narrative approach. And en route quite a lot of basic information, including some modern developments, is imparted. One surprising omission occurs in the section on the deep inelastic electron–proton scattering data Having explained why these very important experiments suggest that the nucleon "is like a pomegranate, not like a jelly", I expected, but did not find, some treatment of the naive quark–parton model. Showing how the data suggests that the "seeds" of the pomegranate have one-third integral charges, would have lent further weight to the subsequently raised hot question of particle physics: do quarks actually exist? Towards the end the narrative style inevitably prevails. But even in the last chapter, on the unified theories of weak and electromagnetic interactions, there is enough to show a (keen) beginner how to embark on his own rudimentary model building.

All in all, I think that the book is remarkably successful, and that it caters well for its intended market. The price too is reasonable by current standards, at least for the paperback edition.

D. Bailin

Organic mechanics

Mechanical Design in Organisms By S. A Wainwright, W. D Biggs, J. D. Currey and J. M. Gosline. Pp. xii+425. (Arnold: London, January 1976.) £8.50.

THIS is an ambitious work which brings together much biological information about structural macromolecules and skeletal tissues with the increasing knowledge of their mechanics. It is a book for the specialist and general reader alike, although comparatively few biologists will be sufficiently familiar with materials science, or adequately equipped mathematically, to follow all the engineering phenomena. The first part deals with various tensile, pliant and rigid materials incorporated into skeletal systems The second part looks into the design of skeletal elements and mechanical support systems, and the third part examines organism-environment interactions. The literature has been assiduously searched and digested, but the book is not an uncritical collection of research results. The authors have rigorously argued their central theme: that structural elements are designed to carry out specific functions in an environment of stresses and strains. Biologists have long appreciated this interplay between form and function, but for many the concept is possibly more an article of faith than a complete understanding. This book provides the comprehension as well as the creeds.

Editorial and technical production reaches a high standard. The book is written throughout in a simple, lucid style (with the occasional touch of humour) making it easy and enjoyable to read. It is elegantly illustrated with numerous drawings and diagrams combining pleasing clarity with meticulous draughtsmanship I detected few errors, but a curious recurrent mistake is the term 'keratin sulphate' which should be keratan sulphate, or keratosulphate. Naturally there are some points of contention. The dogmatic statement (p105) that α chitin alone exists in Arthropoda is some three years out of date: well-defined examples of β and γ chitin are present in some insect peritrophic membranes and larval cocoons. The '9+2' theory for the structure of α-keratin microfibrils is given another airing, but there is no mention of the 'ring-core' model which fits more closely the X-ray diffraction data and computational studies.

Mechanical Design in Organisms is bound to attract and stimulate considerable interest among biologists and others. It will be an important reference book for some time to come, and as a text it will provide a basis for interdisciplinary teaching of new topics in biophysics and biology degree courses

W. Kenchington

Molecular spectroscopy

Theory of Molecular Spectroscopy. Vol 1 · Quantum Mechanics and Group Theory of Vibrating and Rotating Molecules. By C. J H Schutte. Pp. xvi+512 (North-Holland: Amsterdam and Oxford; American Elsevier: New York, 1976) Dfl. 170; $67 95.

THIS is volume 1 of a two volume work and it is not too easy to judge in isolation. It is a strongly mathematical introduction covering vector spaces, matrices, group theory, Hamiltonians in general, both the classical and quantum mechanical form of vibration–rotation interactions, diatomic potential functions and their evaluation, and the interaction of radiation with matter.

The mathematical treatment is thorough and a number of advanced topics are covered, such as the Wilson and Howard Hamiltonian and its modification by Watson, and also the Rydberg–Klein–Rees method of fitting potential curves The treatment is mostly sound and comparatively free of misprints, and the first impression, strengthened by the early chapter on group theory, is favourable. But I became increasingly unhappy as I delved deeper. It is doubtful whether a standard treatment of the hydrogen atom would be expected in an expensive book on rotating molecules And as the text gets to real problems, rather than abstract algebra, a lack of authority becomes apparent. Section 1.4 on normal co-ordinates is especially confused; for example, Schutte's use of Cartesians weighted with the square roots of the masses means that the kinetic energy matrix should be the unit matrix, but this is not recognised in this text And the statement (p241) that the energy "is then ~ kR^2, since k is defined as Nm^{-1}" leads to a lack of confidence as does that on p307· "If a molecule does not have a permanent dipole moment, the selection rules for dipole radiation prohibits the occurrence of an infrared spectrum".

There is a need for a comprehensive advanced treatise on the theory of ro-vibration, but part of the difficulty of writing is to choose the important features and give them once only in a modern and consistent form. This text, which has some good sections, tends to give a rather indigestible slab of everything The price is high and the second companion volume, which will contain the more specific features, is not likely to be less.

D. H. Whiffen

Desert rats

Rodents in Desert Environments. (Monographiae Biologicae, Vol. 28.) Edited by I. Prakash and P. K Ghosh. Pp. xvi+624. (Junk: The Hague, 1975.) Dutch Guilders 180.

THIS is one of several recent books devoted to the adaptations of desert organisms. Most have centred around a unifying disciplinary theme, such as comparative physiology, providing integration. This book of 23 invited chapters focuses on a single taxon of organisms sharing a common habitat Desert rodents, however, are so diverse that appreciating their adaptations requires synthetic interpretation by specialists Although some of the chapters use a synthetic approach (especially those by Newsome and Corbett; Mares; Rosenzweig, Smigel and Kraft; and Ghosh), many are purely descriptive. As a result the value of the book for the general biologist interested in evolutionary and/or ecological phenomena is diminished; the overall quality would have been greatly enhanced by inclusion of a comprehensive, synthetic chapter based on a thorough reading of the invited chapters, indicating those adaptive patterns which are unique to desert rodents and the advantages they offer.

The primary value of the book is that for the first time (and mainly in a single language: English) there are detailed accounts of the biology of rodents from most of the world's deserts, providing a wealth of comparative information on distributions, general ecology and life histories, activity patterns and population fluctuations, behaviour, competitive interactions, and adaptive physiology. The lengthy chapter on Russian jerboas and gerbils by Naumov and Lobachev is particularly welcome. The chapters vary tremedously in quality and scope with some reporting original research or comprising state-of-the-art messages, whereas others reflect woeful neglect of the current literature or are merely rehashes of ideas and materials previously treated. Lapses in editorial rigour are indicated by numerous typographical errors, and occasional misinformation Statements of misinformation I found particularly distressing include: “Tropical Dasyurids such as *Antechinomys* . . . do not normally venture into arid habitats”, p 394 (the only species of these small Australian Marsupials, *A. spenceri* and *A laniger* occur exclusively or primarily in arid habitats); “ . . . Heteromyidae . . . the most common rodents in Arizona and the Sahara Desert.” p 417 (heteromyids are endemic to the new world).

I found the most interesting paper to be that by Rosenzweig, Smigel and Kraft, a state-of-the-art message on coexistence and resource allocation in North American desert rodents. As well as summarising what is known, they emphasise equally what is still unknown about the enormous complexity of competitive interaction Our continuing fascination with desert rodents is well reflected in their statement, “ . . clearly allocation occurs . . . But something that is known to occur ought to have an explanation” (p 251).

To the careful reader this book provides a great deal of information on desert rodents, some synthetic explanations, and stated suggestions or unstated gaps where future research could be productively directed.

Richard E. MacMillen

Drug resistance

Acquired Resistance of Microorganisms to Chemotherapeutic Drugs. (Antibiotics and Chemotherapy, Vol 20.) Edited by F E. Hahn. Pp. xi+272. (Karger: Basel, London and New York, 1976) Sfr 139; DM132; $53.50.

THIS book is a collection of nine chapters by different authors, as follows: Inducible bacterial resistance (Connamacher), Chromosomal mutation to drug resistance in bacteria (Sirotnak); Molecular modifications of anti-microbial agents to overcome drug resistance (Neu); Anti-mutagens and the prevention of chromosomal mutations to drug resistance (Hahn); Genetics of *R* factors (Mitsuhashi *et al*), Molecular biology of *R* factors (Clowes); Experimental elimination of *R* factors (Hahn); Drug resistance in leukaemia (Hutchinson and Schmid), and Drug resistance in the human malarias (Tiggert and Clyde). There is a preface by Hahn.

Although there are valuable data in the volume, the diversity of authors has produced a very uneven presentation; in parts the English is poor in style and even in grammar; and the same data are presented by different authors, not always with the same lucidity or interpretation.

The price of this book—over £29 at the current rate of exchange—is outrageous In view of its uneven quality and the many better sources of information, it is not recommended. If information is needed about plasmids and various other genetic aspects of drug resistance, for example, the reader is advised to consult Falkow's *Infectious Multiple Drug Resistance* (Pion: London, distributed by Academic: New York and London, 1975); for review see *Nature*, **258**, 368 (1975). The two books are of about the same size, but Falkow's treatise is greatly superior and costs only £7.70 ($19.95).

E. S. Anderson

Auger spectroscopy

Photoelectron and Auger Spectroscopy. (Modern Analytical Chemistry.) By Thomas A. Carlson. Pp. xiii+417. (Plenum: New York and London, 1975.) $39.

THE improvements in electron spectrometers and electron detectors which have been achieved over the past two decades have provided new techniques for the study of the nature of the electron orbitals which hold together the positive framework of matter. The three most profitable areas in which these developments have been applied are ultraviolet photoelectron spectroscopy, X-ray photoelectron spectroscopy and Auger electron spectroscopy. The author of this book, who has had long experience in each of these fields, writes about them in a very lively and authoritative manner

After the initial chapters which deal with instrumentation and fundamental concepts the subject matter is divided into: photoelectron spectroscopy of outer electron shells; photoelectron spectroscopy of inner shells, and Auger electron spectroscopy. The first of these topics is mainly concerned with the ultraviolet photoelectron spectroscopy of gaseous molecules and the nature and ordering of the molecular orbitals as revealed by their spectra. The second topic deals largely with the binding energies of the core electrons and how these are shifted by the state of the valence shell environment in which they find themselves—that is, the so-called ‘chemical’ shift In the third—the Auger effect—a beam of electrons rather than photons is focussed on the sample, and valence or core electrons removed in an identifiable manner.

Apart from the importance of the basic information on the electronic structure of matter which these effects reveal the fact that they are of low penetrating power makes them extremely valuable for studying surface phenomena such as catalysis, corrosion, adsorption, and so on.

The book is well referenced and contains a great deal of useful tabulated data It should be valuable both to the general reader and the specialist in the field. **W. C. Price**

Books brief

The Encyclopedia of World Regional Geology Part 1: Western Hemisphere (Including Antarctica and Australia). (Encyclopedia of Earth Sciences, Vol. 8.) Edited by Rhodes W. Fairbridge. Pp. xv+704. (Dowden, Hutchinson and Ross: Stroudsburg, Pennsylvania; Wiley: New York and London; 1975.) $44 40; £22 30

THIS is an unusual and successful book written by some 90 authors. Most contribute one or two articles, although the Editor weighs in with a valiant 60 ranging from Antarctica to the Windward Islands. Oceanic islands are done particularly well throughout. It is a true encyclopedia covering the out of the way as effectively as the well known in contributions extending from a column or two on an atoll, to 140 pages on the US. Long contributions are subdivided so any district can be readily picked out, and regional reviews link accounts of individual countries. Ocean floors are not covered the book sticks to dry land. It will provide basic answers to almost any enquiry requiring knowledge of the geology of a part of the Western Hemisphere. Professor Fairbridge and his colleagues have done a first class job. I look forward to the promised companion volume on the Eastern Hemisphere. **J. Sutton**

Twentieth Century Physics. By Joseph Norwood. Pp. ix+405. (Prentice-Hall: Englewood Cliffs, New Jersey, 1975.) $16.95.

IN this useful book an attempt is made to present in outline our present understanding of the interactions involving matter. The topics covered are special and general relativity, old quantum theory, wave mechanics and atomic, nuclear and particle physics. On the whole the attempt is successful: the author has striven hard to present arguments which are as simple as possible while at the same time powerful enough to enable results to be derived (or, sometimes, made plausible) rather than merely stated. There is a set of problems at the end of each chapter and this is a particularly valuable feature of the book. Unfortunately, the whole is marred by frequent inaccuracies and an informality of style that sometimes degenerates into sloppiness For example, Kepler's Laws are wrongly stated, geodesics in space and space-time are confused, it is asserted that terms in $1/C^2$ are small because their value is 10^{-17} (no units being introduced), and so on. In spite of these shortcomings the book will be useful as an auxiliary text for physics undergraduates. **Michael Berry**

Radio and Microwave Spectroscopy By David J E. Ingram. Pp. 167. (Butterworth: London and Boston, December 1975) £4.50.

THIS is very much a book intended for undergraduates in physics or chemistry and must be judged accordingly. This is not easy for someone who knows more about the topics covered—gas microwave rotational spectroscopy, electron resonance and nuclear magnetic resonance Certainly the language is straightforward, no previous knowledge is expected and the level is appropriate with a text comparatively free of mathematics The examples are largely taken from Dr Ingram's earlier book, *Spectroscopy at Radio and Microwave Frequencies* (1955), and the diagrams do not give a fair view of modern sophistication and one (3.1c) is such a poor photographic reproduction of an oscilloscope screen that it should never have been passed at the galley proof stage. And certainly in 1976 one can no longer maintain, (p109), that for proton chemical shift references "The two protons most commonly used for this purpose are the protons in water . . and the protons in benzene . . .": tetramethylsilane is not mentioned In places, ideas are simplified to a point where explaining more correct views as students advance may be difficult Thus, zero field parameters in triplet states are referred to as "effective" and then allowed to depend on the direction of the zero amplitude magnetic field (p151) This short book is more appropriate for a weak student in a hurry to get an overview than for more able students It is also an example of how difficult it is to write a really good elementary text. **D. H. Whiffen**

Graph Theory: An Algorithmic Approach. (Computer Science and Applied Mathematics: A Series of Monographs and Textbooks) By Nicos Christofides. Pp. xv+400. (Academic New York and London, October 1975) £12.50; $31 00

THIS book presents a comprehensive and up-to-date account of algorithmic development, which has been going on quietly for over 20 years. The problems of optimisation associated with graphs are well known, and the lack of mathematical progress in solving them is universally appreciated What has happened, with the advent of the computer, is the development of some quite powerful and numerically effective methods for solving some of these problems This book expounds, in a clear style and with ample illustration, the detailed steps of the most important algorithms known at present The first four chapters are concerned with the theory of graphs, and with the introduction of ideas important in the numerical study of graphs. The chief topics treated are reachability, connectivity, colouring and set covering. There are chapters on the location of centres and medians, on trees, and on shortest paths. Chapters nine and ten are concerned respectively with Eulerian and Hamiltonian circuits, and there is a detailed discussion of some of the classical problems of operational research mathematics, relating to scheduling and the travelling salesman Chapter eleven deals with flow in networks and the last chapter deals with matchings, transportation and assignment

No practitioner (whether of operational research or of the social sciences generally) concerned with numerical studies can afford to neglect this book. **L. S. Goddard**

Chromatographic Analysis of the Environment. Edited by Robert L. Grob. Pp. x+734. (Dekker: New York, 1975.) $49.50.

CHROMATOGRAPHY is undoubtedly a prime method for the analysis of trace components in the environment, and a good book on the subject would be welcomed. The present multi-authored work starts with an introduction to theory and practice, and follows with 18 chapters covering most of the possible permutations of air, earth, water and waste with gas, liquid, paper, thin layer and ion exchange chromatography. One or two of the chapters are very good but too many are narrowly based and simply catalogue methods—many of them rather old. There is a monumental author index of 38 pages and a very poor subject index, which is the reverse of what is required in a book of this kind. This book fails to give the kind of overall picture of environmental analysis required at this stage in the subject and will mainly be useful to those already expert who wish to have comprehensive reference lists before undertaking development of new methods.

John H. Knox

obituary

Grigorii Sergeevich Pod"yapol'skii, the Soviet geophysicist and human rights campaigner, died on March 8, 1976.

Pod"yapol'skii was born on October 22, 1926, in Tashkent. In 1949, he graduated from the Moscow Petroleum Institute, and from then until 1953 he worked as a geophysical engineer, doing field work in various regions of the Soviet Union (Ukraine, Siberia, Central Asia, the Arctic). In 1953, he began research work at the Institute of Earth Physics.

During the next seventeen years Pod"yapol'skii was mainly concerned with the theory of earthquakes (in particular the statistics of their occurrence and the propagation of shockwaves). In 1969, he spoke at the International Conference on Tsunamis, in Honolulu on the relationship between tsunamis and the earthquakes which trigger them.

Pod"yapol'skii's presence in Honolulu was, in retrospect, somewhat surprising. The composition of Soviet delegations to international conferences is decided more in accordance with the political rather than the academic soundness of the would-be participants By 1969, Pod"yapol'skii was already in official disfavour as a leading member of the growing movement of dissent within the Soviet Union; he was a founder of the Initiative Group for the Defence of Civil Rights in the USSR and a close associate of Academician Andrei Sakharov. His attendance at the Honolulu conference can only be explained, it would appear, by a desire on the part of the Soviet authorities to make a good showing in the international arena, and as an implicit acknowledgement of the high value they placed on his work. This value, however, they would not acknowledge at home. In the same year, 1969, Pod"yapol'skii was prevented from defending his thesis, and the following year he was dismissed from the Institute on the pretext of "staff cuts", so that his scientific career was effectively brought to a halt

According to his fellow dissidents, Pod"yapol'skii was a man of great personal charm, and skilled in reconciling the incipient disputes inevitable among those eager to exercise freedom of speech and opinion. He was also a poet; his work reflecting his scientific and humanitarian interests.

announcements

Appointments

Dr **K. G. Binmore** to the Chair of Mathematics at the London School of Economics.

Professor **Gabriele Veneziano** to the Amos De-Shalit Chair in Theoretical Physics at the Weizman Institute.

Professor **Gerald Holton** as the Mallincrodt Professor of Physics and Professor of the History of Science at Harvard University.

Dr **J. D. Hodge** to the Chair of Botany at Royal Holloway College.

Awards

The **Lenin Prizes** for 1976 were awarded as follows:

to Agasi N. Charakhch'yan, Galina A. Bazilevskaya, Yurii I Stozhkov, and Taisiya N. Charakhch'yan for work on cosmic ray bursts from the Sun and the solar modulation of galactic cosmic rays;

to Nikolai N. Krasovskii, Aleksandr B. Kurzhanskii, Yurii S. Osipov, and Andrei I. Subbotin for work on the mathematical theory of control systems,

to Nikolai N Semenov for work on the kinetics of complex chemical reactions,

to Vladimir S. Sobolev, Nikolai L. Dobrentsov, Vladimir V. Reverdatto, and Vladimir V. Khlestov for work on geological metamorphism;

to Aleksandr S. Spirin, Georgii P. Georgiev, Ol'ga P. Samarina, Murat A. Aitkhozhin, Nadezhda V. Belitsina, and Lev P. Ovchinnikov for the discovery and study of informosomes;

to Sergei V Anichkov and Vasilii V. Zakusov for work on the effect on synapses of physiologically active substances;

to Vladimir I Burakovskii, Leonid A. Bokeriya, and Vitalii A. Bukharin for work on hyperbaric oxygenation in heart surgery;

to Feliks G Arzhanov, Valeri I. Graifer, Valentin V. Karibskii, Aleksandr V. Sinel'nik, Tevgat S. Gainutdinov, and Valentin D Shashin (Minister of the Petroleum Industry) for work on the automation of petroleum production;

and to Vasilii T. Babenko, Anatolii I. Manokhin, Tamerlan S. Shishkhanov, Nikolai P. Lyazhishev, Nikolai P. Slotvinskii-Sidak, and Aleksandr N. Morozov for a new method of producing vanadium.

The National Academy of Engineering Founders Award to Professor **Mason Benedict** for work on nuclear energy.

The NAE Vladimir K Zworykin Award to Dr **C. K. N. Patel** for contributions to laser technology.

The Marconi International Fellowship to Dr **H. Inose** for work on computers.

The Royal Society has elected Professor **S. Benzer**, Professor **W. H. Munk**, Professor **R. W. Sperry** and Professor **C. H. Townes** Foreign Members of the Society.

The Rank Prize for Nutrition to **Norman W. Pirie** for work on the extraction of protein food from herbage.

The Charles Frederick Chandler Medal to Professor **Kai Seigbahn** for developing ESCA.

International meetings

May 18–21, **Drug Receptor and Drug–Enzyme Interactions,** Namur, Belgium (Services des Relations Publiques, Facultés Notre-Dame de la Paix, 61, rue de Bruxelles, B-5000 Namur, Belgium).

May 20, **Onchocerciasis,** London (Betty Harrison, Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London W1N 4EY).

May 27–28, **Oncogenic Transformation,** Lake Placid (Course Secretary, W. Alton Jones Cell Science Center, Old Barn Road, Lake Placid, New York 12946).

July 5–7, **Theoretical Methods in Polymer Physics,** Leeds (Meetings Officer, Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

July 6–8, **Breeding Endangered Species in Captivity,** London Zoo (Dr Michael Brambell, The Zoological Society of London, Regents Park, London NW1 4RY, UK).

July 8–9, **Nutrition and Growth,** Cambridge (Dr J. D. Sutton, Hon Programmes Secretary, National Institute for Research in Dairying, Shinfield, Reading RG2 9AT, UK).

July 12–16, **Properties of Liquid Metals,** Bristol (Meeting Officer, The Institute of Physics, 47 Belgrave Square, London

SW1X 8QX, UK). (Revised date).

July 12–16, **Progress in Marine Research in the Caribbean and Adjacent Regions,** Caracas (Dr H. B. Stewart, Chairman CICAR-II Steering Committee, NOAA Atlantic Oceanographic and Meteorological Labs, 15 Rickenbacker Causeway, Virginia Key, Miami, Florida 33149).

July 12–16, **Catalysis,** London (Sixth International Conference on Catalysis, Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN, UK).

July 13–16, **Psychoneuroendocrinology,** Strasbourg (Professor Paul Mandel, CNRS, Centre de Neurochimie, 11 Rue Humann, 67085 Strasbourg–Cedex, France).

July 27–31, **Transcultural Psychiatry,** Bradford (Conference Secretary, International Congress on Transcultural Psychiatry, Room N2, University of Bradford, Bradford BD7 1DP, UK).

August 4–6, **Applications of X-Ray Analysis,** Denver (Ms Mildred Cain, Denver Research Institute, University of Denver, Denver, Colorado 80210).

August 22–28, **Photosynthetic Prokaryotes,** Dundee (Dr G. A. Codd, Department of Biological Sciences, University of Dundee, Dundee DD1 4HN, UK).

September 3–7, **Annual Convention of the American Psychological Association,** Washington DC (APA Public Information Office, 1200 Seventeenth St, NW, Washington DC 20036).

September 7–9, **Wind Energy Systems,** Cambridge (Organising Secretary, WES, BHRA Fluid Engineering, Cranfield, Bedford MK43 0AJ, UK).

September 28–30, **Cellular Aspects of Neoplasia,** London (Dr G. Hodges, Imperial Cancer Fund, Lincoln's Inn Fields, London WC2A 3PX, UK).

March 30–April 1, 1977, **Mixing,** Cambridge (Deadline for submission of synopses · June 30) (Organising Secretary, 2nd Mixing Conference, BHRA Fluid Engineering, Cranfield, Bedford MK43 OAJ, UK).

July 4–8, 1977, **Rhythmic Activity of Fish,** Stirling (J. E. Thorpe, Freshwater Fisheries Laboratory, Pitlochry, Perthshire PH16 5LB, UK).

July 18–23, 1977, **Physiological Sciences,** Paris (Pr J. Scherrer, Secrétariat du XXVIIe Congrès International des Sciences Physiologiques, UER Pitié-Salpêtrière, Cedex 1300, F-75300 Paris-Brune, France).

August 10–19, 1977, **History of Science,** Edinburgh (Date for preliminary registration: June 30) (Dr Eric Forbes, Congress Secretary, XVth International Congress of the History of Science, The Royal Society of Edinburgh, 22 George St, Edinburgh EH2 2PQ, UK).

September 5–10, 1977, **Social Insects,** Wageningen (Dr J. Beetsma, International Agricultural Centre, Lawickse Allee 11, PO Box 88, Wageningen, The Netherlands).

October 3–7, 1977, **Small-Angle Scattering of X-Rays and Neutrons,** Gatlinburg (Dr R. W. Hendricks, Metals and Ceramics Division, Oak Ridge, National Laboratory, PO Box X, Oak Ridge, Tennessee 37830).

Person to Person

As a tribute to **Rene Moricard** a prize of 125 000 B.F. will be awarded for experimental work relating to the initial stages of cancer of the uterine cervix. Manuscripts should be submitted by December 1, 1976 to Dr R. Vokaer, Clinique Obstétricale et Gynécologique, Hôpital Universitaire Brugmann, Place Van Gehuchten, 4, 1020 Brussels, Belgium.

Four bedroom contemporary house in Lexington, Mass. (6 miles from Cambridge), with use of community swimming pool and car included, offered Jan.–July 1977, in exchange for similar or smaller house in Oxford. G. Wolf, 56-235 M.I.T., Cambridge, Mass. 02139, USA.

If you receive or produce a newsletter on biological research, please write giving details of title, publisher, frequency of publication, subject content and subscription rate to Trevor Crane, COBI Research Officer, c/o Prof. J. B. Jepson, Middlesex Hospital Medical School, London W1P 7PN. This information is being sought for the Committee of Biological Information (COBI) so that a list can be compiled. The list will aid research workers in contacting others with similar interests, provide access to another source of information and possibly indicate topics for current awareness bulletins (macroprofiles).

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office The section will include exchanges of accommodation, personal announcements and scientific queries We reserve the right to decline material submitted No commercial transactions

Reports and Publications

Other countries

New Zealand. Ministry of Transport New Zealand Meteorological Service Meteorological Observations for 1974—Stations in New Zealand and Outlying Islands, Including the Cook Group, Tokelau Islands, Niue Island and Western Samoa. Pp. 104. $1.50. Rainfall Observations for 1974—Stations in New Zealand and Outlying Islands, Including the Cook Group, Tokelau Islands, Niue Island and Western Samoa. Pp 73 $1 (Wellington · NZ Meteorological Service, 1974.) [261

CERN—European Organization for Nuclear Research. CERN 75–18 Radiation Damage to Electronic Components By S Battisti, R. Bossart, H. Schonbacher and M. Van de Voorde Pp. 29. (Geneva CERN, 1975.) [261

United States Department of the Interior · Geological Survey Bulletin 1394–H · The Whitehorn Granodiorite Of the Arkansas Valley in Central Colorado By Chester T. Wrucke. Pp iii+8. (Washington, DC · Government Printing Office, 1974) 25 cents [261

World Health Organization Technical Report Series. No. 581 · Nonproprietary Names for Pharmaceutical Substances—Twentieth Report of the WHO Expert Committee. Pp 32. Sw. fr 6 No 582: The Epidemiology of Infertility—Report of a WHO Scientific Group. Pp. 37 Sw. fr. 6. No. 583 Pregnancy and Abortion in Adolescence—Report of a WHO Meeting. Pp. 27. Sw. fr. 6. Promoting Health in the Human Environment. (A review based on the Technical Discussions held during the Twenty-seventh World Health Assembly, 1974) Edited by Evelyn E Meyer and Peter Sainsbury. Pp. 69. Sw. fr. 12. (Geneva · WHO, London HMSO, 1975) [261

World Health Organization. WHO Offset Publication No. 22: Mental Health Services in Developing Countries. (Papers presented at a WHO Seminar on the Organization of Mental Health Services, Addis Ababa, 27 November to 4 December 1973) Edited by T. A. Baasher, G. M. Carstairs, R Giel and F. R. Hassler. Pp. ii+132. (Geneva. WHO, London · HMSO, 1975.) Sw fr. 18. [271

Department of Meteorology, University of Helsinki. Report No. 8 Diagnostic Studies on the Intraction Between the Time-Mean Flow and the Large-Scale Transient Fluctuations in the Atmosphere. By Eero Holopainen. Pp. 14. (Helsinki. Department of Meteorology, The University, 1975) [271

World Health Organization. Technical Report Series, No 580 · Control of Nutritional Anaemia with Special Reference to Iron Deficiency—Report of an IAEA/USAID/WHO Joint Meeting. Pp. 71. (Geneva: WHO, London. HMSO, 1975) Sw. fr. 7. [281

Carnegie Institution Annual Report of the Director of the Department of Terrestrial Magnetism, 1974/1975. (Reprinted from *Carnegie Institution Year Book 74.*) Pp 109–301. (Washington, DC: Carnegie Institution, 1975.) [281

Science Council of Canada. Report No. 24: Technology Transfer—Government Laboratories to Manufacturing Industry. Pp 61. (Ottawa Information Canada, 1975.) $1.20. [281

Comptes Rendus des Travaux du Laboratoire Carlsberg. Vol 40, No. 14 Ultrastructural Studies of Meiosis in Males and Females of the c(3)G17 Mutant of *Drosophila melanogaster* Meigen By Soren W. Rasmussen P.p 163–174 Dkr. 11.50. Vol 40, No. 15 Respiratory Rate Through the Growth-Division Cycle of *Acanthamoeba* SP. By Kirsten Hamburger Pp 175–186. Dkr. 11 50. Vol. 40, No 16 Characterization of a Protein-Rich Beer Fraction by Two-Dimensional Immunoelectrophoretic Techniques. By Jorn Hejgaard and Steen Bech Sorgensen. Pp 187–204. Dkr. 11.50 Vol. 40, No 17: An Apparatus for Cultivation of Cell Suspensions in Continuous Flow Cultures By K. H Cohr and P. B Suhr-Jessen. Pp. 205–213. Dkr. 11.50. (Copenhague. Carlsberg Laboratorium, 1975) [291

Australia Council of Scientific and Industrial Research. Report of the Division of Horticultural Research, 1973/1975. Pp. 137. (Adelaide. CSIRO, 1975) [291

Koninklijk Nederlands Meteorologisch Instituut. Mededelingenen Verhandelingen No 95 · On the Theory of the Electromagnetic Seismograph By I Csikos. Pp 82. No. 96: Oceanography of the Ria deA rosa, N.W. Spain. By L. Otto. Pp. 210. (De Bilt Koninklijk Nederlands Meteorologisch Instituut, 1975) [301

Great Britain

What's Science to History or History to Science? By Margaret Gowing. (An Inaugural Lecture delivered before the University of Oxford on 27 May, 1975) Pp. 25. (Oxford: Clarendon Press; London · Oxford University Press, 1976) 85p net. [301

The Radiochemical Centre. Technical Bulletin 75/5. Radioactive Reference Materials for Nuclear Medicine Pp 24. (Amersham: The Radiochemical Centre, 1975.) [301

Ministry of Agriculture, Fisheries and Food Fisheries Radiobiological Laboratory Technical Report FRL 10: Radioactivity in Surface and Coastal Waters of the British Isles, 1972/1973 By N. T Mitchell Pp. 40. (Lowestoft Fisheries Radiobiological Laboratory, 1975) [301

Determination of the Rates of Clearance of Insoluble Compounds of Plutonium from the Lung By Lynda Watts Pp. 8. (Harwell, Didcot National Radiological Protection Board, 1974) [301

Government Statistical Service. Government Statistics · a Brief Guide to Sources. 1976 Edition. Pp 19 (London. Central Statistical Office, CO. CSO Section, Great George Street, SW1, 1975.) *gratis.* [22

Philosophical Transactions of the Royal Society of London B · Biological Sciences. Vol. 273, No 924 A Discussion on the Biosynthesis of Porphyrins, Chlorophyll and Vitamin B_{12} Organized by A. Neuberger and G. W Kenner Pp. 75–357 + plate 1. UK £15 10; Overseas £15 60 Vol. 273, No. 925. The Climbing Species of *Ficus*—Derivation and Evolution By E. J. H Corner. Pp. 359–386. UK £1.50, Overseas £1.55. (London · The Royal Society, 1976.) [52

nature

May 20, 1976

Is the Sun being oversold?

THE United States will spend £36 million in 1976 on solar energy research and development. Japan will spend £18 million, France and West Germany £3 million each, and the European Economic Community £2 million. Meanwhile expenditure in the United Kingdom will be less than £500,000. It that the right level, and if not, what is? The UK section of the International Solar Energy Society (ISES) has just published a major report (*Solar Energy: a UK assessment*, available from the Royal Institution, 21 Albemarle Street, London W1, 375pp, £10) which looks at prospects in the UK, attempts to predict the future for solar energy and tries to outline a research and development programme.

The Sun can be used for a variety of energy-related purposes: although heating applications with flat-plate collectors and electricity generation with solar cells are the best known, the report carefully outlines many other prospects. There is simply better building design, there are agricultural and biological applications of photosynthesis, including energy plantations and there are photochemical reactions; and within each of these areas there are many ingenious schemes, some of which work, many of which might, given appropriate investment of time, money and (dare we say it) energy.

The report is optimistic. It is supposed to identify areas particularly worthy of support, but after reading it one is left wondering whether anything is not worth supporting. Projects that are near to fruition should be encouraged; projects that are rank longshots should be encouraged; projects that are particularly suitable in Britain should be encouraged, projects with export potentials should be encouraged. Of course with nearly 40 scientists, engineers and civil servants collaborating enthusiastically in the report, it was unlikely that anything but an advance-on-all-fronts view would emerge, but even so the report does seem to suffer from a weak link in moving from scientific statements to policy recommendations. It is sometimes difficult to link the recommendations made at the end of each chapter to any very clearly enunciated argument within that chapter, and one suspects that the phrase "this technique could have export potential" has been added too often to have retained its credibility.

By the year 2020, the report argues, solar power could provide 35 million tonnes of coal equivalent of energy annually in the UK. It is almost impossible to estimate accurately what fraction of total energy consumption this might represent; it represents roughly 10% of present UK annual consumption. But elsewhere the report postulates that the contribution is 10% of 2020 needs, which is different, maybe by a factor of two. And one ever-optimistic Fleet Street correspondent persists in reading the figure as 30%. The report is not strong on its projections; it sometimes claims figures which are difficult, if not impossible, to find, but insofar as the projection of 35 million tonnes means anything, it probably implies that by 2020 up to 20% of all houses would be fitted with solar water heaters, and that there would be a significant contribution to our electricity supply from solar cells. (Solar energy could, of course, contribute in many valuable ways to isolated, local needs well before 2020.)

Do these predictions ring true? On the heating side they require, for instance, that in the next twenty years 0.5% of all present housing stock should be fitted with solar heaters each year. At a charitable £300 per installation and with the knowledge (not perhaps wide enough at present) that solar heating does not eliminate the need for a conventional heating system, one can only describe this prediction as hopeful. On the solar-cell side, the assumption has to be made that present costs of cells can be cut by a factor of a hundred to bring costs per watt into line with those prevailing in other electrical generators. The evidence for such a prospect seems tenuous in view of the lack of any spectacular progress up to the present in reducing costs. Further, the severe problem of cheap electrical storage will have to be confronted; the report rather lamely asserts that since the problem is receiving a great deal of attention and no fundamental scientific barrier is known to exist, "a breakthrough in the reasonable future may be expected". The same can presumably also be said of nuclear fusion.

And if solar cells experience all the required cost reductions, the land issue will have to be faced. To produce 10% of our 2020 total energy requirements from solar cells would require between 1 and 2% of the land area of Britain to be covered with solar farms (roads at present occupy a similar percentage). This is not a trivial fraction; the report asserts that the problem is "unlikely to be acute for many years as the use of solar energy will only develop slowly". But if and when solar farms do come, the size of the problem will be much greater than that of building superhighways through the country. To say, then, that solar energy is "particularly attractive in physical environmental terms" is simply to put off the day of reckoning.

The impression that the report leaves behind us is of a good survey of the science, but a loose job on the policy. This leaves the proposals for more national expenditure on research and development very exposed. ISES calls for £2 million in 1976, rising to £8 million by 1980 and £20 million in 1986. Very crudely, this would compare, in the early 1980s, with British plus EEC expenditure on fusion research. There is a real danger that over-optimism now may cause resistance to even a fraction of such money being made available later. ☐

International science and the Third World

NOTHING is ever done in this world until men are prepared to kill one another if it is not done. So said Shaw. Whether or not the United Nations offers a failsafe mechanism good enough to prove him wrong (it probably doesn't), the issues its agencies discuss receive too little attention. True, the UN may be ineffectual. It may be over-bureaucratic. But it is one of the few highly visible structures addressing itself to the "big" issues.

That, in fact, may be another of its problems. Whatever distance governments now put between themselves and those they purport to serve, it is but a miss compared to the mile between the UN's "big" concerns and the everyday experience of the millions whose problems it wishes to solve. But there it is —recently, as the United Nations Environment Programme (see opposite) addressing itself to persistent if now less fashionable "eco-issues"; last month as the United Nations Law of the Sea Conference, tackling questions about economic zones and deep sea mining; right now, as the United Nations Conference on Trade and Development discussing the transfer of technology, commodities and debt problems.

If there is a common thread to all this, it concerns the ever more fragile relations between the developed and underdeveloped countries. The problem of commodities, which bears directly on the world food problem (see page 181), and questions about the transfer of technology, encapsulate the principle issues.

But they all remain "macro" issues. If re-presentation in "micro" form is now necessary, the plea below, from a scientist in Lesotho, is a useful start in the area of science.

Lesotho is one of the poorest countries in the world. Poverty links the "macro" and the "micro" issues. And poverty, to quote Shaw once again, is the greatest of evils, and the worst of crimes.

Accommodating the Paradisian wilderness

Professor A. Brock of the Department of Physics at the National University of Lesotho in Southern Africa, draws attention to the position of the scientist working in the Third World

"PARADISIA and Dominatia: Science and the Developing World", the title of an article by Michael Moravcsik and John Ziman which appeared last year in *Foreign Affairs* (**55**, 699–724; 1975), struck so many familiar notes that, as a scientist working in a less developed country, I feel impelled to bring it to wider attention.

The problems are those of the individual scientist and of the small scientific community in a typical third world country—Paradisia; the relationship to be explored is the one between Paradisia and the powerful metropolitan country, Dominatia, in whose sphere of influence it once lay, in the context of science and technology, rather than that of politics, culture or economics. Science and technology are almost always viewed as desirable goods by Paradisians, and to this end the basic sciences are taught in the schools and universities, and modern technologies are adopted in industry and agriculture.

The picture of scientific poverty and intellectual isolation will be all too familiar to anyone who has visited or worked in Paradisia. Expatriate scientists will also know the feeling, but they usually enjoy the benefits of regular visits to Dominatia, and often have a network of contacts built up before they left for Paradisia. The Paradisian scientist, on the other hand, has fewer opportunities of this type because he cannot qualify for "home" leave, and his contacts in Dominatia derive mainly from his days as a research student rather than from a period of mature scientific practice For him the keyword is isolation.

The concern here derives from the view that science flourishes through the efforts of individual scientists, but only in the context of an active scientific community which provides many channels (both official and unofficial) for encouragement, criticism, review, stimulation, interaction and publication. This is the rich soil in which science grows. But in Paradisia the scientific climate is semi-arid and the soils desert-like.

Take, for example, the keen Dominatian scientist on secondment to Paradisia, prepared to improvise in the absence of technical facilities, but handicapped beyond measure by the arrival of *Phys. Rev.* a year late and the absence of colleagues to discuss his work with. His productivity, it is suggested, will rapidly decay under these circumstances. A more personal example: as a palaeomagnetist, I have been fortunate to find my material in Paradisia—fresh Precambrian lavas, or richly fosilliferous Plio-Pleistocene lake sediments. But my results take shape in correspondence with colleagues in Canberra, Stanford, Berkeley or Leeds, rather than over the tea table in my own laboratory How much more difficult for a Paradisian scientist forced by circumstances to rely almost exclusively upon his own resources. And what a triumph for those who manage to produce substantial scientific work.

The task of helping Paradisian scientists working under such circumstances is more difficult than that presented by more normal aid programmes, in which buildings or equipment or funds are provided for some specific purpose, such as a soil science laboratory, or a natural resource survey, or a feasibility study. The task is that of trying to create and maintain indigenous scientific activity in Paradisia. The suggestion is that the world scientific community bears some responsibility towards its less fortunate members in Paradisia, by extending to them some of the advantages that are taken for granted in Dominatia. Lavish funding *per se* is not envisaged here, although it is worth remarking that even the most heavily "squeezed" research budget in the UK would look lavish to a Paradisian.

It is the opportunity for full and free participation in the scientific processes of discussion and exchange, the feeling of "belonging", that Paradisians particularly lack. Topics such as the provision and distribution of journals, the suitable placement of research students, attendance at conferences, the exchange of staff, academic visits, are suggested as areas in which careful attention to the needs of Paradisia would pay dividends. The development of an active lively scientific community is a lengthy process, and cannot be the object of any single aid programme.

One suggestion, though, is a device for opening a range of scientific appointments in Paradisia to young scientists from Dominatia as a normal stage in a research career. Another is that free copies of the major scientific journals be sent to all accredited scientific institutions in the less developed countries. What a blessing this would be to "out of touch" scientific workers. These suggestions apart, however, the concern should be to alert the world scientific community to the general plight of the small fraction of its members in Paradisia, and to suggest that there is much that can be done at the individual as well as the institutional level to help. □

Two steps forward, one step back

The United Nations Environment Programme seems to be faltering. **Richard Sandbrook** reports on the efforts to deal with environmental problems on a global basis

THE United Nations Environment Programme (UNEP) has been having an uncomfortable time of late. Not only are funding problems now severely hampering the agency; there also appears to be increasing dissent about the work programme, both within and without the Secretariat. Nothing new in one sense for the UN; but at a time when many states are taking a new look at the UN in general following the recent "group of experts" report on its structure, and when its major donor, the United States, is severely pruning budgets, uncertainty in UNEP is not going to help its causes.

Initially UNEP was set up to carry through the 109 recommendations that came out of the UN Stockholm Conference on the Human Environment held in 1972. It was structured to consist of a Programme, which is the outline of the projects being tackled by UNEP, other UN agencies, national governments or non-governmental organisations; the Environment Fund, into which states make voluntary contributions; the Secretariat, consisting of 100 professional staff; and a Governing Council, composed of 58 UN nations, which meets annually to review the programme and state of the fund. In addition, the executive director of UNEP chairs the Environment Coordination Board, which includes the heads of all other agencies in the UN family.

The fund started out with pledged contributions of $110 million to carry it through its initial five years, and the energetic services of Maurice Strong, its first executive director. Strong had left his mark by the time he departed in January. He had tried hard to dispense with many of the usual UN nonsenses that slow up the whole business of running the existing UN agencies. Contracts were handed out in an almost commercial manner—rather as if a new research and development programme was in the making—and initially he circumvented the delaying process of UN recruitment by appointing consultants. The relationships between UNEP and non-governmental organisations, governments and other specialised agencies was—and still is—fluid and open. The programme itself was neatly compartmentalised into priority levels and clear-cut objectives.

But UNEP had its fair share of teething problems too. Nairobi, the home of the programme, is not as accessible as the more "traditional" Geneva and New York, a particular disadvantage since the whole venture was set up partially to get existing outfits, such as the FAO, UNESCO and WHO, to cooperate together through the newly created Environment Coordination Board. Mr Strong and his team spent many months in the first three years commuting between the world's capitals for planning and promotional meetings. Back in Nairobi permanent staff recruitment was very slow, and the planning of programme projects became delayed. By the 1975 governing council meeting the fund was carrying forward large surpluses and certain states were complaining that not enough was being spent; UNEP had too much government money in its bank earning interest. Ironically this now forms the central excuse of the US Government for delaying their "promised" contributions (initially a 40% slice of the first five-year $110m).

Changing attitudes

Attitudes to the "environment problem" also began to change. Strong was above all a superb salesman who generated great interest and concern before Stockholm and immediately after it. In the early 1970s "the environment" was an important issue for western politicians. Now, some four years on, the environment issue is clearly not as politically pressing as it was; and, besides, many governments would argue that national pollution control is advancing rapidly. Initially the less developed world was very suspicious of the whole affair. Strong's answer was to promote "eco-development"—or "environment and development"—and a challenging declaration that united these themes was developed (the COCOYOC Declaration 1974). Gradually a constructive relationship built up between UNEP and the doubting Third World nations.

UNEP, however, has never been seen as a route for aid or technical assistance in the eyes of the major western donor countries—it is much more a "catalyst" (the most used word by delegates to the recent 1976 governing council) for sound environmental management. The idea of UNEP growing into an enlightened development agency with funding capabilities is certainly not acceptable. All this leaves the impression that the idea was potentially good; but for some the fashion is now passing, while for others UNEPs objectives are becoming less relevant. The current acting executive director, Dr Tolba, is nobly conducting a holding operation, but eventually the big issues will have to be resolved.

The first and major issue to be sorted out is the leadership question. Dr Tolba, an Egyptian, is due to leave this year; he could emerge as the new man from the horsetrading that goes on in New York, but the OECD countries and the South Americans may put up a challenge. Then there is the problem of replacing before the end of the year Mr Steadman, who runs the fund, and Mr Munro, who directs the programme. Both are available for office for only a short period. UNEP has always been beset with changes at the top, and this has created uncertainty all the way down.

Financial problems

The second issue is the state of the fund. The long lead time on project approval in the formative years has pushed the peak cash needs of UNEP toward the end of its first quinquennium. The cash requirements double between 1975 and 1976 to $40 million and then slowly climb to an annual requirement of $55 million by 1979. (It is worth noting that the US Federal outlay in 1976 on pollution control and abatement was $683 million for research and development activities alone.) Currently, the agency predicts a shortfall of some $10 million to $12 million a year from 1977 onwards. This figure is based on the revised pledges known at March 15 this year, some of which are well below those originally given when the agency was established. Commenting at the governing council in April, Tolba said that only $41 million of the pledged $109 million had so far been paid up, and some of that was in non-convertible currency from the USSR. The US was singled out as the major turncoat. Special visits to Moscow and probably the US are planned, but matters look bad: some 42 projects, Tolba reported, had already been delayed because of the cash crisis. Things could conceivably become a little easier for the agency after the US elections.

The third problem concerns the dream of Stockholm itself: how effective it will be in practical terms will in turn affect the long-term support UNEP gets. To date UNEP has successfully translated a few good ideas, such as that of the FAO for a Mediterranean pollution convention, into actual events (Barcelona, March 1976). They have also been closely involved in planning and funding the UN Water

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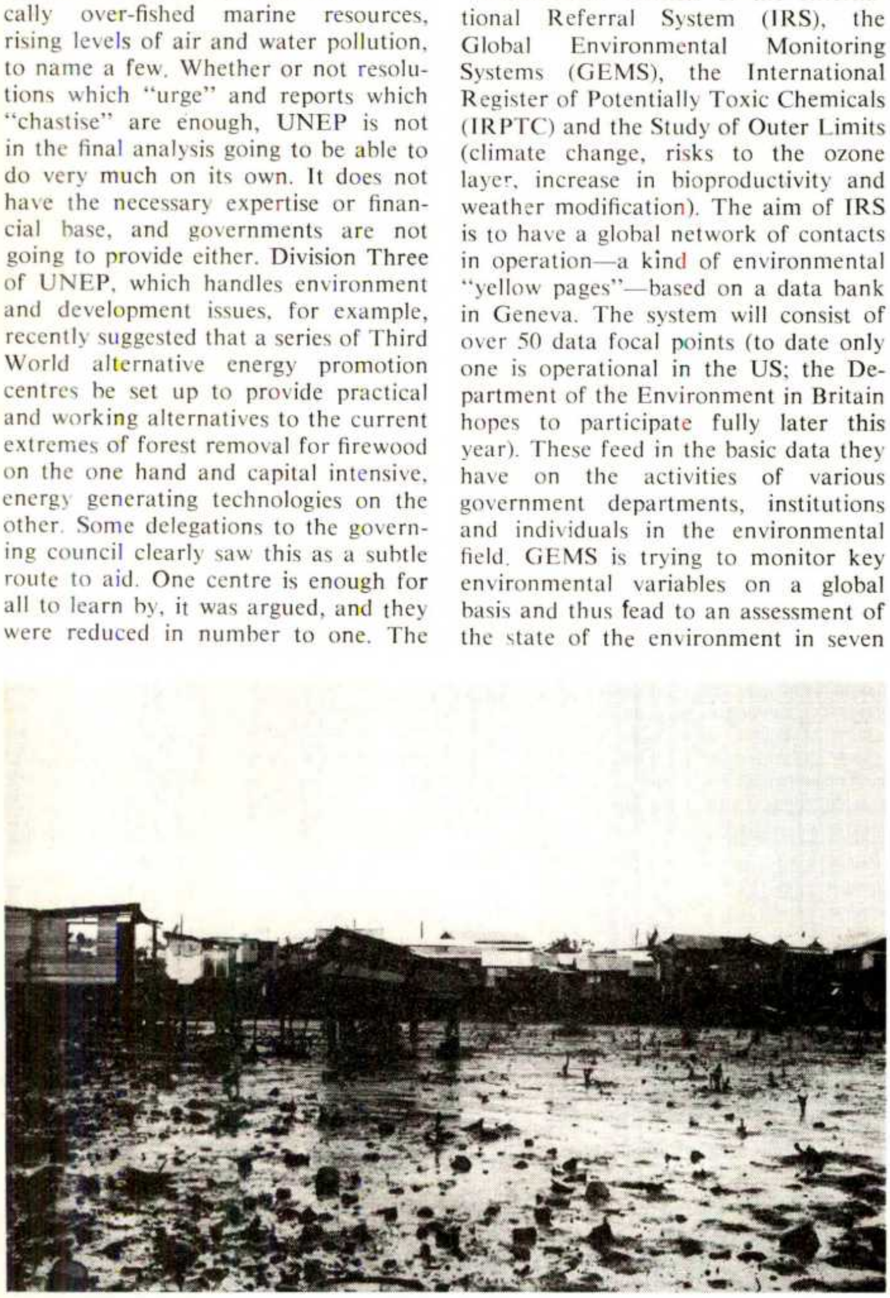
Conference (March 7, 1977, Argentina), the UN Deserts Conference (August 29, 1977, Nairobi), and the imminent UN Human Settlements Conference (May 31 to June 14, Vancouver). Much credit must go to UNEP for causing the latter two events to happen at all. All these conferences will help establish priorities and routes for aid and technical assistance in the coming years on pressing development fronts.

Tasks remain

But UN conferences can only assist in the evolution of "global environmental management"; all too often they seem to result in little more than high sounding cliches. Substantive tasks remain: the world's disappearing rainforests, the protection of the ever-growing number of threatened species, the chronically over-fished marine resources, rising levels of air and water pollution, to name a few. Whether or not resolutions which "urge" and reports which "chastise" are enough, UNEP is not in the final analysis going to be able to do very much on its own. It does not have the necessary expertise or financial base, and governments are not going to provide either. Division Three of UNEP, which handles environment and development issues, for example, recently suggested that a series of Third World alternative energy promotion centres be set up to provide practical and working alternatives to the current extremes of forest removal for firewood on the one hand and capital intensive, energy generating technologies on the other. Some delegations to the governing council clearly saw this as a subtle route to aid. One centre is enough for all to learn by, it was argued, and they were reduced in number to one. The idea of doing it regionally was ruled out.

Earthwatch is, in fact, the only significant functional task that UNEP is involved in apart from its conference support work. The remainder consists of overscanning some 150 projects to be carried out by other UN agencies, governments and institutions. These are organised into some eight priority areas, as follows: Human settlements and human health (1976 Budget $6.3 million), Ecosystems research (1976 $8.19 million), Environment and development ($3.6 million), Oceans ($3.6 million), Energy ($550,000), Natural disasters ($200,000), Environmental management ($450.000), Environmental law ($300,000) and support functions ($5.91 million). Earthwatch takes $3.4 million this year.

Earthwatch consists of the International Referral System (IRS), the Global Environmental Monitoring Systems (GEMS), the International Register of Potentially Toxic Chemicals (IRPTC) and the Study of Outer Limits (climate change, risks to the ozone layer, increase in bioproductivity and weather modification). The aim of IRS is to have a global network of contacts in operation—a kind of environmental "yellow pages"—based on a data bank in Geneva. The system will consist of over 50 data focal points (to date only one is operational in the US; the Department of the Environment in Britain hopes to participate fully later this year). These feed in the basic data they have on the activities of various government departments, institutions and individuals in the environmental field. GEMS is trying to monitor key environmental variables on a global basis and thus fead to an assessment of the state of the environment in seven distinct areas. These include atmosphere monitoring in relation to climate, ocean monitoring, the depletion of renewable resources, natural disasters and human health. Throughout there is much emphasis on the concept of baseline studies.



More than just an environmental problem: the litter and decay of a forgotten community (WHO photo)

Coordination only

UNEP is the first to point out that much of this monitoring is already done by other agencies and that its role is one of coordination only. There is, for example, UNESCO's Man and the Biosphere Programme; the work of the World Meteorological Organisation on climate; the FAO's work on land use, fisheries and soil structure; and, of course, many governments have their own monitoring programmes. It is hoped that variables will be monitored around the globe in a uniform and comparable way, and the data collated into central locations. But the problems in practice are going to be considerable. So-called baseline studies in the marine environment, for example, are notoriously difficult to carry out, and to many are of dubious scientific value anyway. To determine the quantities of various pollutant involved from atmospheric and land sources and their effect on the biological system is an almost impossible task; the state of the initial biological system is usually not known, is probably inherently variable, and the pathways of the pollutant far from clear.

It is thus apparent that UNEP structures are again in a state of wait and see. Until the financial and leadership questions are cleared up its future direction and style are unclear. At present, it is prevented from being the radical outfit that the Third World want by the priorities of its powerful western backers. Any confrontation with the real forces that lead to environmental degradation — inequality between nations and over-indulgent living as Mr Strong once put it—must wait. As the executive director stated in April, four years after Stockholm every country has come to grips with the fact that sustained development and meaningful growth were impossible without a commitment to preserve the environment and promote the rational use of resources. But if the Secretariat has adopted the principles of the brave new world, its major funding source has not. And yet, leaving the development issue aside, there is a clear need for UNEP simply to coordinate the significant effort being made toward the protection of the biosphere at a national, regional and international level. It has gone a long way in convincing governments and international agencies that this should be done. It would be a great pity if it were now denied the wherewithal to do it. ☐

When enough is not enough

Howard Rush, Pauline Marstrand, John Gribbin and Gordon MacKerron look at what is sometimes called the world food paradox

WITHIN the past few months a series of publications* has again focused attention on the complex of problems underlying the "world food crisis". These analyses aim at different audiences, but all have a message in common: our inability to feed the world's population is not due to physical factors placing limitations on total world food production. They also recognise that the causal factors in world hunger and malnutrition are the enormous economic, social and political problems associated with poverty.

The key physical factor, of course, is availability of land. Most recent estimates are based on calculations published in 1967 by the US President's Science Advisory Committee (PSAC), suggesting that the maximum area which would be cropped is 6,600 million hectares, of which about half has been cultivated at some time and about 1,400 million hectares harvested in any one year. Although other estimates—such as those by FAO and the US Department of Agriculture—have produced a broad spread of figures, the PSAC estimates are roughly in the middle of the range, and all investigators agree that only about half of the potential area is now used. The estimates differ partly because of different assumptions about the feasibility of obtaining water, but they all show that apart from competing uses of land, existing world food production could be roughly doubled without improved technology.

Lester Brown and others have postulated that increasing costs of development will preclude utilisation of much of this land—an assumption also built into the *Limits to Growth* model—but there is room to query this assumption. Although it is true that the tendency is for the range of available technologies to move towards the very capital intensive (and expensive) end of the spectrum, in principle developing countries can still select from alternatives ranging down to such labour intensive projects as the human muscle used to build the Yangtse dam. There is certainly room here for further debate, partly because future cost assumptions depend so heavily on the nature and pace of future technological change.

Using the PSAC estimates, the maximum population supportable has been taken as 7,200 million at existing standards of nutrition, or 157,000 million on a 1967 "Japanese" diet. Although it is commonly stated that 400–500 million people are starving in the world today, it seems unlikely on the basis of WHO estimates that more than 60–70 million are currently so severely undernourished that they are using bodily protein reserves (muscles, including heart muscle) as a source of metabolic energy.

The important factors

This represents less than 2% of the present world population, and is probably a smaller proportion than ever before, but it is nothing to be proud of; the efforts made over the past three decades to improve food supply to the hungry have beeen successful in part and are worth sustaining. But if physical factors are not critical in determining the availability of food to the world's population now and in the immediate future, what are the important factors—and are they really so enormous as to provide insurmountable difficulties?

The key to the world food "problem" is now seen to be poverty, not population. Poverty within nations means that poor people receive a smaller share of the food available nationally, while poverty between nations means that the poorer nations have less food per head to share in the first place. And the inequalities are greatest in the poorest nations, so that the smallest slices of the cake are themselves divided the most unfairly.

Between 1951 and 1971 the global production of cereals more than doubled, while the world's population "only" increased by 50%. More than half of the increase in food, however, was absorbed by the richest 30% of mankind, so that the extra food was shared almost equally between 1,000 million people in the developed countries (DCs) and the 2,500 million people in the less developed countries (LDCs). This pattern emphasises that the food is there—or can be produced—for those who can afford it. It is because they are poor that the hungry—whether they be nations or individuals—are unable to buy sufficient food, or to develop their farming sufficiently to provide for their own needs. Under the present world economic system, their hunger, as such, is largely irrelevant; "demand", in the food market as in other markets, means ability (or willingness) to pay. As in other free markets, the rich have a dominant influence: their power in the market distorts demand patterns, and allows them to obtain the greatest benefits for themselves. One important result is that in the poor nations—as well as the rich—increasing demand for meat prevents the most effective use of available land by diverting large areas of cereal from human to (inefficient) animal consumption.

It was a reduction in this market demand that led to a reduction in planted cereal acreage in the developed countries during the 1960s, at a time when there was clearly an increasing need for food among the hungry poor who had no financial access to, or influence on, the market. Between 1960 and 1970 the LDCs were increasing food production at least as fast as the developed countries, but the increasing market demand for "luxury" food—beef, dairy produce and so on—forced prices upwards, including the prices of cereals used for feeding animals as well as people. Together with the emphasis at government level in many LDCs on cash crops that could be sold to developed countries to boost exports (coffee, sugar and so on) this had serious effects on the poor in the LDCs, where even if luxury foods were being exported essential foods had still to be imported in bulk (as represented in Table 1).

The shortages of the early 1970s and high prices have been blamed on many natural factors, not least the weather

Table 1 Changing Patterns in the Grain Trade (million metric tons)

Region	1934–38	1948–52	1960	1966	1973*
North America	+5	+23	+39	+59	+91
Latin America	+9	+1	**	+5	−3
West Europe	−24	−22	−25	+5	−3
East Europe & USSR	+5	**	0	−4	−27
Africa	+1	0	−2	−7	−5
Asia	+2	−6	−17	−34	−43
Australia, New Zealand	+3	+3	+6	+8	+6

Plus sign indicates net exports; minus sign net imports
*Estimated **Not available
Source: *Financial Times*

**Future World Trends,* UK Cabinet Office (April 1976); *The World Food Crisis and Third World Development: Implications for UK Policy,* UK Select Committee on Overseas Development (February 1976); *Profits of Doom,* C. Robbins and Javed Ansair (War on Want, January 1976); *World Food: A Political Task,* Howard Wagstaff (Fabian Research Series 326).

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(which, in fact, was not so much bad as simply worse than the previous, exceptionally good, years of the 1960s). But these shortages, although real, were not physically necessary; they resulted from the control of the market by producers in the developed countries.

LDCs can only pay for the food they import with the foreign exchange earned by their exports; ironically, agricultural produce makes up three-quarters of those exports, but the LDCs' share of the world market in these products is falling as the developed countries produce more and increase their domination of the market. According to War on Want, "In 1966, prices for exports from the DCs were 13% higher than in 1958 while prices for exports from the LDCs fell by 11% . . . more and more has to be exported to buy the same goods from the DCs". The resulting imbalance, with the developing nations' debts to foreign countries increasing substantially faster than their GNP, means that in the majority of cases the gap between debts contracted and the means of repaying them is widening every year. As this gap increases so do the inequalities between developed and developing nations.

Internal factors

The responsibility for this, however, does not lie solely with the developed nations' control over world markets. Their market control does place the poor of developing nations at a severe disadvantage, but the inequalities are heavily reinforced by agricultural policies and local price factors within the developing nations themselves, where agriculture accounts for more than a third of total production and rural regions provide about three-quarters of the total population.

These regions and most of their impoverished population have little say or control over their economy. The majority are landless or own less than a single hectare of land. What little agricultural policy there is in many LDCs, including investment programmes, is heavily biased in favour of the prosperous minority. The result of this imbalance is clearly seen in the example of India, where 7.7% of the population own more than 50% of the head. More often than not the situation is made worse by national development policies which stress industrialisation of urban areas dependent on the agricultural sector for resources.

As Keith Griffin has pointed out, the outstanding feature of the agricultural sector in almost all LDCs is the bias in access to factors of production (that is, land, credit, water, fertilizers, technical knowledge, etc.) towards the prosperous landowners. Government policies supported by the economic and political influence of this minority virtually control the market structure and allocation of resources—thus assuring no shift in the *status quo* detrimental to their interests.

Technical change, once heralded in the form of the "green revolution" as the solution to man's hunger, becomes a further aid to the rich because of their dominance of the world system. Technical change which relies on easy access to credit and often substantial inputs of water, fertilizer and pesticides, may well increase production—but only for the minority of landowers in whose favour the market is biased. Any innovation under the control of the *status quo* tends to strengthen the *status quo*. Agricultural innovation has become widespread, but the result has not only been increases in productivity but also increases in inequality; the rich get richer, but the poor get poorer.

In recent years the world's food supply has increased faster than the rate of population growth. As suggested by nearly all recent analysis, physical factors are not critical to feeding the world's population. But the fact that fewer people need face starvation than previously believed does not reduce the seriousness of the difficulties which remain: the problems are now recognised as those of poverty and politics. It might seem that these are no less insurmountable than the physical restrictions often assumed—although we could feed a much larger population, it may require a social revolution to remove the inequalities at the root of poverty. As the UK Select Committee on Overseas Development stressed in its report, "the main answer to the world food problem is to give those who are hungry the means to feed themselves, or the income to buy food". ☐

USA

Antidisestablishmentarianism

Arrangements by which the US President receives advice on science matters have now been institutionalised. Colin Norman reports from Washington.

N.E.W.S. photo

President Ford signs the science policy bill, watched by (left) Senator Frank Moss, chairman of the Senate Space Committee, and Representative Olin Teague, chairman of the House Committee on Science and Technology.

WITH storm clouds hovering overhead, and with many elder statesmen of the scientific community in attendance, President Ford last week held a small ceremony in the White House rose garden to mark the signing into law of a bill re-establishing a science policy office in the White House.

The event was pure ceremony. Contrary to rumours flying around beforehand, Mr Ford did not nominate anybody to head the office—the director will also be the President's Science Adviser—nor did he indicate how he intends to use the office when it is eventually set up. Instead, he simply took the opportunity to make a few obligatory remarks about the importance of science and technology in helping to meet "the challenges and opportunities which lie ahead for this nation and the world", and signed the bill with "great pleasure". Fortunately, the rain held off.

The ceremony nevertheless marked an important event in the annals of science policy. The bill, which had been championed by numerous prominent scientists and also by Vice-President Nelson Rockefeller, essentially reinstates the science policy apparatus dismantled three years ago by Mr Nixon. Moreover, since the office has now been established by an Act of Congress—rather than an act of Presidential pique—to remove it.

Though scientists, of course, played a prominent role in shaping national policy during the Second World War, they didn't have a permanent place in the White House until 1957, when President Eisenhower acquired a full-time science adviser during the post-Sputnik panic which swept the country. The adviser was made chairman of the President's Science Advisory Committee, a panel of scientists drawn from academic and industry. President Kennedy established the Office of Science and Technology (OST) five years later, as a small White House operation headed by his science adviser, and that apparatus remained intact until 1973. President Nixon then suddenly scrapped it, largely because he disagreed with much of the advice it was offering him, particularly on such matters as the need for an anti-ballistic missile system and the SST programme. When Mr Nixon abolished OST, he gave the head of the National Science Foundation the extra job of being science adviser to the White House.

The bill signed by Mr Ford last week will establish a small Office of Science and Technology Policy (OSTP) to advise the President and other White House bodies on matters involving science and technology. It also establishes a committee of scientists to conduct a two-year review of the federal government's science and technology programmes, after which the committee can be kept in business as an advisory unit if the President so desires. White House officials say that President Ford hopes soon to nominate somebody as his science adviser, but it will take several weeks to get the office under way.

It should be noted that the establishment of the office couldn't have come at a much worse time. Having suffered a series of staggering defeats in primary elections these past two weeks, Mr Ford's tenure in the White House must be considered precarious, at best. Thus, with a change of Administration at least on the cards a few months after OSTP is established, nothing dramatic should be expected from the office for some time. ☐

THE Senate last week followed the lead of the House of Representatives by voting to cut off federal support for a controversial research project designed to assess the effects, if any, of marijuana on human sexual behaviour. The Senate's action will ensure that the project will be aborted, and thus for the first time Congress has reached deep into the scientific peer review process and cut off a research project for political reasons.

The project, a $121,000 two-year investigation which was to have been conducted by Dr Harris Rubin at Southern Illinois University, had been approved as scientifically meritorious by a committee of behavioural scientists, endorsed by a top level government advisory committee, and supported by a panel consisting of the federal government's most senior health officials. Congress voted to stop the project, however, because some powerful members have labelled it morally unacceptable and scientifically trivial.

The Senate, at least, held a short debate on the matter before deciding to shut the project off. Senator William D. Hathaway, Democrat from Maine, offered an amendment to restore the funds, arguing that "the real issue is the integrity of a carefully constructed government-wide mechanism for awarding research grants and contracts". He argued that by voting to deny the funds, "possibly the least expert group of federal employees to gather in one building—the US Congress—seems now to have taken upon itself the role of grand inquisitor with regard to scientific research". Hathaway's amendment was easily defeated, however, on a voice vote.

It's not too surprising that, once the issue was raised, Congress should vote to eliminate Harris's project. It is, after all, an election year, and Congressmen would be reluctant to defend a vote in favour of spending tax payers' money on a project involving marijuana and sex. But, as the Federation of American Scientists (FAS) noted in a statement last week, the votes set a worrying precedent. "'Every controversial project that finds its anti-champion can now be expected to be raised" in Congress, FAS suggests.

● After months of debate by several committees of scientists and a week of conflicting rumours, the Food and Drug Administration (FDA) formally announced last week that it has no intention of lifting its controversial ban on the artificial sweetener cyclamate. Citing a number of "unresolved safety questions", FDA Commissioner Dr Alexander Schmidt said that he has asked Abbott Laboratories, the manufacturer of cyclamate, to withdraw its petition seeking to restore the sweetener to the market. If the petition is not withdrawn, Schmidt said he would simply reject it.

Cyclamate was banned in 1969, on the basis of studies which suggested that it increased the incidence of bladder tumours in rats when fed to them in high doses over prolonged periods. The FDA ban initiated a chain of similar actions in other countries.

The FDA's decision to keep cyclamate off the market is, however, based more on questions of general toxicity than on the shaky evidence that cyclamate is a carcinogen. A panel of scientific experts, assembled by the National Cancer Institute, reported last February that "although the present evidence does not establish the carcinogenicity of cyclamate", a number of studies have raised worrying questions. In short, the evidence for carcinogenicity is equivocal.

Schmidt said last week that he is worried about "unanswered questions" concerning cyclamate's potential for causing damage to reproductive organs, causing chromosomal damage, and elevating blood pressure.

A spokesman for Abbott has said that it is unlikely that more safety tests will be conducted to resolve those questions, and it is therefore unlikely that Abbott will renew its petition. FDA's decision thus could be the final word in the long battle over the sweetener.

● Mr Jimmy Carter, the leading candidate to be the Democratic Party's Presidential nominee, last week called for a voluntary, worldwide moratorium on the sale of uranium enrichment and nuclear fuel reprocessing plants, including those sales already negotiated. Speaking at a conference at the United Nations Centre, Carter said that the threat of nuclear weapons proliferation from such sales is too great for "business as usual". He called for an un-sponsored world energy conference to discuss worldwide energy problems, and he said that "there is a moral imperative that demands a worldwide effort to ensure that if we travel down the nuclear road we do so with our eyes open".

The nuclear powers, he suggested, should provide more leadership in preventing the spread of nuclear weapons. For a start, he suggested that the United States and the Soviet Union should agree on a five-year moratorium on weapons tests, during which they should negotiate a complete nuclear test ban. The recently negotiated threshold test ban, he said, is "wholly inadequate".

As for the US nuclear power programme, Carter suggested that it should be kept to "the minimum necessary to meet our needs", and it should have to meet "much stronger safety standards".

CANADA

Spraying controversy revived

A link is suspected between the use of certain tree sprays and the condition known as Reye's syndrome. David Spurgeon reports from Ottawa

THE spraying of forests in the Maritime provinces to prevent infestation by the spruce budworm has created a sharper controversy than ever this spring because of the deaths of five children from a disease suspected of being linked to the insecticides used. It is not the first time arguments have arisen over the spraying programme: DDT was used as an insecticide in the 1950s and 1960s until it was found to have caused severe fish kills in surface waters. But it is the first time human deaths have been linked to the programme.

A number of children, mostly from areas that have been heavily sprayed in the past, have died from Reye's syndrome, a condition first described in 1963 following the US Air Force's aerial defoliation in Vietnam and Thailand. The disease causes increased susceptibility to viral infection, and it is the spray dispersal emulsifiers which are suspect; the sprays used are Fenitrothion and Phospamidon.

The current budworm outbreak, which now covers about a third of the province of Nova Scotia, was first reported in 1969. The budworm (*Choristoneura fumiferana*) is a moth that lays its eggs in firs and spruces in July. The eggs hatch in about 10 days, and the larvae spread through the trees. They remain dormant for the winter, emerging in late April and early May to begin feeding on new shoots. In June they enter the pupal stage, and the moths which emerge in June or July can defoliate and kill trees to the extent of causing their death.

Massive increases in population can occur when there is a large incidence of host trees and favourable weather. This has happened in the Maritime provinces six times since the early 1700s. Predictions for this year were bad from the point of view of the forest industries, particularly in Cape Breton Island, so Novia Scotia Pulp Ltd and Nova Scotia Forest Industries—a branch of Stora Kopparbergs Bergslags AB of Sweden—applied to the Nova Scotia government for permission to spray with Fentrothion or Phospamidon.

The Nova Scotia department of lands and forests recommended against it, but the Cabinet gave approval in February. Seven weeks later, on April 2, Cabinet approval was withdrawn as a result of a story that appeared first in the *Cape Breton Post* and subsequently in other newspapers. The story summarised the work of a research team at Dalhousie University begun in 1974 which indicated that a link was possible between the spraying programme and the deaths from Reye's syndrome. It was based on material given to the *Post's* editor by a PhD student who was a member of an environmentalist group.

The research group had published a preliminary report on their work in *The Lancet* in 1974, and has a further paper in press now. Its members applied spray chemicals topically to the abdomens of about 13,000 mice in the same concentrations used in the spraying programme, and then exposed the animals to a virus. They found that the mice died, not as a result of the virus or of toxin but of some third entity. At the time they published in 1974, they had applied only the insecticides as sprayed, to the animals. Later they purchased the carrier substances separately and concluded that one of the emulsifiers used in the sprays was probably the substance linked to Reye's syndrome.

Dr J. F. S. Crocker, one of the research group and an assistant professor of paediatrics, emphasises that the research has been strictly with animals and indicates only that a link between the spraying programme and the deaths from Reye's syndrome "is possible theoretically." The group is now planning *in vitro* experiments and work with primates, and has proposed identifying chemicals in the tissues of children struck by Reye's syndrome—if possible before they succumb. Dr Crocker himself finds it extraordinary that so little research has been done in this field. He says that if the companies that do the spraying make up their own spray formulation, they do not even require a licence for the emulsifiers, whereas the spray mixture must be licenced if it is packaged as a ready-made formulation. Yet the chemical structure of the emulsifiers is unknown—and of course the effects on humans are unknown.

The budworm problem has been worst in New Brunswick in past years, and has only recently spread to Nova Scotia. No spraying has actually been carried out in Nova Scotia, but in New Brunswick it has always been carried out by Forest Protection Ltd, a Crown corporation representing the provinces and its major pulp and paper industries. Decisions about whether to spray or not in a given area have been made by the corporation's board of directors and approved by federal and provincial governments. Costs have been shared equally by the federal government, provincial government and industry. Fenitrothion has been the chief insecticide used since 1968: it is claimed to be non-persistent, non-cumulative and not subject to concentration in the food chain. There is also evidence it is less harmful to fish than is DDT.

This year's spraying programme in New Brunswick will cover 10.2 million acres. The federal government has opted out of the programme, but the federal department of regional eco-

Canadian cutbacks

THE Royal Society of Canada has joined in the general clamour of protest by Canadian scientists against a federal freeze on research funds. The Prime Minister, M Pierre Trudeau, recently received a brief signed by W. Bennett Lewis, president of the council of the Society's Academy of Science, which said the 1975–76 level of federal support for government laboratories was only 83.3% of the 1969 level in terms of the fraction of federal budgetary expenditures. Research in Canadian universities is in an even worse position, with support in 1975 reduced to 67% of the 1969 level—and although the decline was arrested last year, the freezing of grants this year will cause support to decline to about 60% of the 1969 level.

Although it was recognised in 1969 that government support of industrial research and development was already too small, it is now even smaller, the brief said. In terms of the fraction of federal budgetary expenditures it had fallen by 1975 to 68% of the 1969 level. To make matters worse, the Department of Industry, Trade and Commerce had abolished one of its aid-to-industry research and development programmes and had cut back another.

Tables and graphs accompanying the brief showed that Canada was alone among advanced nations in its reductions in support for science and technology. Organisation in other industrialised countries with functions similar to those of the National Research Council of Canada (supporting in-house research and development and university grants and scholarships) had been treated almost as well, and in some cases better, than they were in 1969.

nomic expansion will contribute. As a result of the controversy in Nova Scotia, questions have been asked in the New Brunswick house about the advisability of carrying on with the programme. In an article to be published in the June issue of *Science Forum*, Neal Benneworth and Chris Bailey contend that spraying should be prohibited because it is "an energy-intensive attempt to bend nature to conform with the forest industry's narrow concepts of how a forest should behave".

They say the operation only increases in the long term the chances of occurrence of what it was originally intended to prevent, because preservation of the host trees by spraying "simply improves the environment for the budworm and increases the likelihood of further population expansion." This is so because the forest and the budworm are a self-regulatory system: the budworm destroys the forest and ensures a new development of host species favourable for future budworm generations. The present composition of the forest, they say, has evolved as a result of budworm predation removing mature balsam fir preferentially, which allows less competitive species of trees to survive. Budworm spraying merely protects the host trees, which are economically significant to the forest industry, and this in turn improves the environment for the budworm, thus increasing the likelihood of further population expansion. ☐

USSR

One of the obstacles to communication between Soviet geologists and their foreign colleagues in recent years has been the unwillingness of Soviet geologists officially to admit the possibility of continental drift. This block was not due to any political considerations—unlike those imposed in Stalin's time on genetics and cybernetics—but seems to have been due to personal opposition from certain leading geologists, notably Professor V. V. Belousov of the Institute of Earth Physics in Moscow. Recently there has been a discernible relaxation in this attitude: the popular science magazine *Priroda* has carried an article dealing with this "hypothesis", and even Professor Belousov himself has somewhat half-heartedly discussed the subject in his latest book.

Now, however, a TASS radio bulletin, transmitted in English and hence clearly for international consumption, has given even greater approval to this concept, stating that Australian rock samples, collected by M. Ravich of the Institute of Arctic Geology in Leningrad, have proved to be "completely identical" to samples from the Antarctic opposite Australia, thereby giving added proof of the "so-called theory of Gondwanaland". According to Professor Ravich, the samples match not only in chemical composition and age (2,000 million years), but also in the size of the deposits, and give credence to predictions of mineral wealth in the Antarctic similar to deposits in Australia, South Africa and South America. A number of Soviet geologists have privately supported the concept of continental drift for some time. The TASS broadcast indicates a possible reversal of the official view of the Soviet geological establishment.

- The chance a private individual in the USSR has to participate in a major scientific research project nowadays is extremely limited. But it was granted to the citizens of Byelorussia, the Baltic States and the Leningrad-Novgorod region of the Russian Republic by the flight, on February 11 this year, of what has been named the "Baltic bolide".

This large meteorite, which appeared in the twilight over the Gulf of Finland, crossed the gulf moving in the general direction of Moscow before burning up. Its flight time of more than 100 seconds, instead of the normal one second or so, is a record for any meteor, and is attributed to a velocity comparable with that of the Earth and a trajectory virtually tangential to it. Before the bolide finally broke up with a fine display of natural pyrotechnics, some 600 people following it along its path had observed it closely enough for their letters and sketches to help astronomers at the Pulkovo observatory plot its course. According to V. Kratt, the observatory's Director, the bolide ceased glowing only at a height of 25 km, and there is reason to hope that some fragments may have penetrated the atmosphere to ground level. The Soviet Academy of Sciences has therefore appealed to all persons living along the route to survey the area for fragments.

Cam & Pen Int.

- The State Committee for Science and Technology of the USSR's Council of Ministers has signed an agreement on technical cooperation with British Petroleum, to run for an initial period of five years. It provides for broad-based reciprocal scientific and technical cooperation on a long term basis, and is the result of some two years of negotiation, since Academician Vladimir A. Kirillin, the committee's Chairman, visited Britain in 1974.

The agreement envisages exchanges of scientific and technical information and of specialist staff, joint research and development, the organisation of symposia on topics of mutual interest, and the joint implementation of research programmes and projects—on the production of unicellular proteins from petroleum wastes, for example. The State Committee has shown considerable interest in purchasing the expertise for deep-water drilling developed by BP in connection with the Forties field in the North Sea.

One new Soviet development in anti-pollution measures, however, is unlikely to form part of any exchange agreement. According to Moscow radio a new agent for cleaning up oil spills in the Caspian has been discovered: the cockle *Cerastoderma*, which, although only some 2.5 cm long, filters up to 15 litres of water through its body in its feeding process. Any oil contained in this water is coated with slime and eliminated, forming a sludge which falls to the sea-bed, where, it is thought, it is later rendered harmless biologically.

- The latest volume of the Large Soviet Encyclopedia carries a brief, 75-word biography of Andrei Sakharov, complete with photograph, describing him as a "Soviet physicist and Academician" and recording that he was thrice awarded the title of Hero of Socialist Labour (the highest Soviet civilian award). Sakharov's human rights work is nowhere mentioned: his recent activity is dismissed with the remark that "in recent years he departed from scientific activity". Sakharov himself commented that he was "very pleased" with the entry.

Vera Rich

UK ENERGY

BRITAIN'S "Save It" energy conservation campaign clearly doesn't apply to the increasingly protracted debates on the subject itself. Not that the energy spent discussing the issues is entirely wasted, even though it certainly looked that way three weeks ago when the latest rush of events was about to start.

What research and development was in hand, a backbench MP asked, and what new technology developed, so that alternative sources of energy could, if economic, be rapidly exploited? Mr Anthony Wedgwood Benn, Secretary of State for Energy and Britain's principal advocate of open government, was simply uninformative:

> Considerable research and development is in hand on indigenous raw materials which are or might be sources of energy. Much of this work is carried out by the nationalised energy industries, the United Kingdom Atomic Energy Authority (UKAEA), the nuclear industry and the oil companies. In the field of new non-nuclear energy sources, my Energy Technology Support Unit has carried out assessment studies. Research and development programmes are being drawn up for those areas which show most promise of making a significant contribution to the country's energy needs. The United Kingdom agencies also participate in international energy research programmes, such as those of the European Communities and the International Energy Agency.

Just three days later Mr Benn's department launched a major research programme on wave power.

It was an unjustifiably inauspicious start to a period of great activity on the alternative energy front. Whether the MP was anticipating the announcement is not known. If he hoped to probe behind the veil shielding the department's Energy Technology Support Unit (ETSU), he did not have too long to wait before another attempt was made. Three top men from ETSU gave evidence the following week to the Energy Resources Sub-committee of the House of Commons Select Committee on Science and Technology. They offered little hint of the content of the Department of Energy's proposed research and development programme for existing and new sources of energy—that had to wait another week, until the Department of Energy's Chief Scientist, Dr Walter Marshall, appeared before the committee on behalf of the Advisory Council on Research and Development for Fuel and Power (ACORD). ACORD has now passed on that programme to Mr Benn.

But ETSU was not totally unforthcoming. It confirmed that it was placing contracts on behalf of the department and was thus playing a supervisory, monitoring role as well as performing its specified tasks on energy sources and energy conservation. ETSU also revealed its preferential ordering of alternative energy sources: wave power offered the "best potential contribution" and looked the most promising; solar came next, ahead of geothermal; wind and tidal power together took up the rear.

Without some sort of weighting, it all seemed rather crude. The subsequent ACORD hearing indicated that ETSU's chief concerns have been solar, geothermal and wind power; wave power studies were the province of the National Engineering Laboratory, while the Central Electricity Generating Board investigated tidal power. Obviously there is close collaboration, not least through the ubiquitous Dr Marshall and a plethora of committees. ACORD, in particular, is crucial. With members drawn from the nationalised fuel and power industries (coal, gas and electricity), from the oil industry, from research bodies (the SRC and the UKAEA) and from the academic world, it considers the results of ETSU's technical studies and advises Mr Benn on both research and development needs and the size of appropriate programmes drawn up by steering committees.

Thus it was able to confirm ETSU's preference to the select committee: it had considered tidal power (April–May 1975), and ordered a limited study of technical issues relating to the Severn Barrage; wave power (June 1975), which produced the £1 million feasibility study launched at the end of last month; geothermal energy (October 1975), any research programme on which needs to be integrable with the EEC's work in the area; wind energy (December 1975), about which a decision was deferred pending further study of costs; and solar energy (February 1976).

It was, in fact, solar energy's turn to capture some attention by this time, thanks to the publication of a massive document by the UK section of ISES, the International Solar Energy Society (see page 177). The bullish tone it adopts about solar energy prospects contrasts with the apparent view of ACORD. ACORD "accepts in principle the need for a national R & D programme" and wants a more detailed study, backed by research and development, "to define the options more precisely"—in other words, the case for solar energy, at least as presented to it by ETSU, is not yet absolutely proven.

Whether all this reflects a genuine clash of views over priorities will probably only emerge after the one-day "national energy conference" organised by Mr Benn for June 22; ACORD's recommendations to Mr Benn are to be made public the same day. The 50 organisations invited to this more obvious example of open government include representatives of industry, the unions, consumer, conservation and environmental interests, and professional, academic and research bodies. In the meantime, the ISES document is an attempt to reinforce the case for solar energy. Clearly the circle of interests that the subject of energy now covers is widening fast.

So fast, in fact, that the issues involved in alternative energy seem to be growing increasingly political—partly thanks to Mr Benn, but principally because the potential of the various energy sources under scrutiny turns less on the well documented scientific evidence that can be brought to bear than on the impenetrable economic factors involved. The ISES document provides just one example: the case it puts for solar energy is based on more than mere scientific feasibility, but it is not really couched in the propagandist terms typical of a document urging policy changes. As one environmentalist privately pointed out, though, it does provide excellent ammunition.

Alternative energy, however, is but the tip of the iceberg, as any table of comparative expenditures on energy in Britain would quickly show. And as if to add to the recent excitement, the Energy Research Group at the Open University has churned out its own critique of the electricity industry, advocating a national fuel policy and suggesting that sheer overestimation of demand has produced a large measure of overcapacity. It can't be long before the fact that power is consumed to such a large extent in the form of electricity also becomes a legitimate area of enquiry.

Back in the committee rooms of the House of Commons, however, was the poor Science Research Council (SRC), also giving a view on alternative energy resources—the beleaguered Sir Sam Edwards was in the chair. He took the opportunity to cock a snook at the Treasury: in an obvious reference to the condition of "big" science under the SRC, he indicated that even if it was available extra money would not be devoted to alternative energy.

IN BRIEF

Explosions treaty delay

Work has been completed on the final draft of a US–USSR treaty limiting the size of so-called peaceful nuclear explosions, but President Ford has delayed signing the treaty to keep it from becoming an issue in some key Republican primary elections. Formal signing ceremonies, which were to have been held last week in Washington and Moscow, were dropped when Mr Ford suffered a string of defeats at the hands of Ronald Reagan, his right-wing challenger. Anxious not to give Reagan yet another foreign policy issue to snipe at, Ford decided to delay the signing until after the crucial May 18 Michigan primary. The irony in all this, however, is that so far the treaty has been attacked almost solely by liberals who regard it as a sham in terms of arms control.

Nuclear trade moves

US pressure for the severest curbs on the sale of nuclear reprocessing and enrichment technology is expected when the "Group of Seven" (now swollen to ten) nuclear powers meets secretly in London next month. Reports indicate that US delegates will move to dissuade West Germany and France from going ahead with plans to export to Iran and Pakistan. They may also press for a cancellation of the West German–Brazilian deal agreed last year. Meanwhile, Congress is expected to approve a request from the American General Electric Company to export two nuclear installations to South Africa.

New USSR group

A new dissident group, headed by physicist Yurii Orlov, has been formed in Moscow. The group aims to monitor the implementation in the Soviet Union of the Helsinki pledges on human rights. Dr Orlov was quickly arrested and informed that he and the other members of the group would be liable to legal action if they persisted with this "unconstitutional activity". Released after an hour, Dr Orlov stated that the group would continue to function.

Shooting the rabids

KENNETH MELLANBY

Our authorities are right to be concerned about the risk of rabies being introduced into Britain. In recent years the disease has spread rapidly over Europe, and has now reached the coast of France just across the English Channel. All dogs entering the country have to spend 6 months in quarantine so those already infected may be detected; the main risk, however, is thought to be that a pet dog incubating rabies might be smuggled into England, either on a small boat which eluded the customs or, tranquillised, in the boot of a car. Our officials are increasingly alert to the danger, and heavier penalties are being given to those caught trying to evade the law, but the risk cannot be completely eliminated. It is also possible that rabies could reach Britain by other means. We do not have vampires, which are notorious carriers in Central America, but even the apparently harmless insectivorous bats, which are known to fly across the channel, are able to carry the virus. It would even be possible for a fox to swim the channel.

In France foxes have been the main carriers of rabies, and would probably be the greatest danger in Britain. There are therefore plans to kill all the foxes living in an area around any focus of rabies which might occur. Now that the fox is becoming a common suburban denizen in Britain, its potential as a disease carrier is increasing. So is the task of control if this becomes necessary. Foxes likely to endanger city dwellers live in overgrown cemeteries and disused railway embankments, and their elimination will be very difficult. On the other hand in rural areas, where foxhunting is popular, there should be less trouble. Contrary to the view put out by supporters of foxhunting, there would be little difficulty in killing off every fox in their territories in a few days using gas, shooting and poison. Foxhunting is only a method of culling the surplus population, and mainly serves to conserve the species in many areas. The sport would have to be stopped if rabies appeared in the countryside, as the hounds and, in turn, the hunters, would be at risk if they chased a rabid fox.

Wildlife conservation would suffer greatly if rabies became a serious problem in Britain. Not only foxes but many other animals, particularly carnivores like stoats and weasels, might be a danger, and there would be attempts to exterminate them. As there is already considerable pressure on these species in Britain, further persecution would probably be effective, and the whole balance between predators and their prey would be upset. The result might well be an unwelcome population explosion in undesirable species like the rat. The effect on the countryside might be serious.

So far our preparations in Britain have been to stockpile vaccine and serum, to protect animals and to treat humans should rabies appear, and to make plans to try to wipe out foxes and other wild animals. Surely we should immediately devote our attention to our domestic pets? Although an infected fox might appear unnaturally tame, and infect a child (particularly one interested in wildlife) who played with it, a rabid dog would clearly be a much greater danger. While I would not wish to interfere with the right of the responsible pet lover to keep a dog, I think that we should take immediate action to eliminate the large numbers of uncared for, stray and feral dogs which are already a real danger to children and even adults in many parts of the country.

It is estimated that less than half the six million dogs in Britain are licenced, even though the fee remains ludicrously low at 37½ pence. These dogs will be more difficult to eliminate than foxes, so we should start at once. At present there is no requirement to licence a puppy under six months old, so many pets are bought for children only to be turned out when they become larger, hungrier and less attractive. Hundreds of thousands of animals are abandoned when their owners go on holiday. A vigorous campaign to destroy all unlicenced dogs, and a much more expensive licence, required even before a puppy could be acquired, would go far to solve the problem Even without the risk of rabies a reduction in the number of unwanted dogs would make our cities cleaner, more hygienic and safer—and would cut down the need for expensive imports of high protein dog foods which might reduce hunger in other parts of the world.

correspondence

Class structure in science

SIR,—To be castigated in a leader column is for me a unique and not entirely pleasant experience, but one which, given the choice, I would rather have had for something I did say than for something I did not say.

In talking to the Research and Development Society on April 27 I was careful to say that I did not believe simple direction was the right way to harness the resources of university science to our national objectives. I made no suggestion that (to quote your leader) "research funding in universities should be approved only if the research has clear economic benefit". Nor did I say that "individual universities should be designated as centres of expertise for certain industrial research purposes".

I emphasized that to benefit more directly from university science was essentially a matter of changing the traditional attitudes towards research of other than the very pure kind, and said in this connection that it "would help, perhaps, as a conditioning mechanism, if all applications for funds for academic research were required to state what would come from its successful completion, with the words 'a better understanding of' or any paraphrase of those words banned". No question here of "clear economic benefit" or approval based on such a concept—just a suggestion that clear thinking about where a project fits into the scheme of things would help the attitude-changing process. My mention of banning the phrase "a better understanding of" was a half-humorous reference (well understood by the audience, in which research management was fairly strongly represented) to the fact that such a phrase is the resort of nearly all woolly proposal writers.

In suggesting that we might consider allocating a major interest area to each academic institution I was not thinking of industrial research and made no mention of it. The examples I gave of the kind of theme institutions might tackle were very broad divisions of our national life and the objects of the suggestion were

- to ensure that all aspects of our communal existence were being thought about
- to generate groups of people of different disciplines sharing a common interest in an important national theme.

A journal like *Nature* which is looked upon as the epitome of scientific accuracy has a special duty to be certain of its facts. If there is a class war in science (which I doubt), your role, Sir, is that of *agent provocateur*.

I presented my talk not as a means of expounding specific proposals but to help in generating serious discussion on a vital topic. Your handling of it will almost certainly be prejudicial to that objective, but if any of your readers would like to know what I actually said I shall be happy to send them a copy. I will even send one to you, Sir, if you are interested in fact rather than fiction.

M. K. MCQUILLAN

Tewkesbury, UK

Frozen mineral waters

SIR,—In his article (March 18, page 182) Allan Piper makes it clear that, as soon as the exploitation of the mineral wealth of Antarctica becomes a commercial proposition there will be a major political conflict. Surely the way to handle this situation is to tackle the problem now, while the commercial issues are still relatively remote. Yet I am very much afraid that the temptation will be to postpone any resolution of the situation until the problem arises, by which time it will, of course, be too late to avoid direct confrontation and very probably military intervention.

I believe that action is necessary now to defuse this situation; for example by an agreement to sign over all mineral rights on the continent to the United Nations Organisation It is to be hoped that signatories to the Antarctic Treaty will not sidestep the issue at their meetings this year and next.

J. A. EADES

University of Bristol, UK

Allergic reactions

SIR,—Reports that animal handlers are allergic to laboratory strains of rats, mice and rabbits are becoming increasingly prevalent (Correspondence, March 25, page 280). In the pages of *Nature* and *Science*, the problem has been considered only from the human point of view; we bemoan the fact that many of our colleagues must either walk around with gauze masks or gulp antihistamines in order to survive their experimental subjects. We suggest that the time has come to view the increased number of allergic reactions from the murine rather than human perspective. The rat is evolving; we are the catalysts speeding its evolution.

Consider an animal handler approaching a colony of rats, to some of whom he or she is allergic. Is it not natural for the handler to select for experimentation only those animals who do not cause an allergic reaction? Faced with a roomful of rats, who of us would willingly choose the rat which caused our eyes to tear, our nose to drip or our bronchi to constrict. We are selectively destroying non-allergenic animals leaving behind the allergenic to act as breeders. One can see additional problems arising in the future. Rats and mice are usually picked up by the tail. Just as selection for super-allergenic strains increases the rat's chances of survival, so does selection for the short-tailed or tailless rat.

If we must root around in the rats' cage for five minutes just to find his tail, we suspect that we'll either move to the next cage or suffer a bitten finger. In short, the super-allergenic, tailless rat is a problem only for the experimenter; from inside the cage it will undoubtedly be greeted with chuckles or cheers.

JACK A. KORNBLATT
MARY JUDITH KORNBLATT

Concordia University,
Montreal, Canada

Human anatomy

SIR,—Your correspondent Anthony B. Harris (May 6th, page 10) describes his "experiments" on young ladies purporting to determine a functional basis for structural asymmetry. His results, which suggest to him a positive correlation between handedness and asymmetry, are at complete variance with our observations on a thousand normal individuals, although asymmetry is certainly the rule rather than the exception.

Since Anthony Harris' measurements are all of circumference or volume, this may account for his inability to detect equivalent asymmetries in the two-dimensional representations of the human form to be found in the National Gallery; these asymmetries, however, are observable in sculpture of many centuries and cultures.

R. C. CONNOLLY
P. H. DANGERFIELD

University of Liverpool, UK

news and views

H-2 and HLA sequences

from J. C. Howard

ONE of the most important and illuminating observations in the convoluted history of the major histocompatibility complex (MHC) was the demonstration by Snell, Klein and Shreffler that the highly complex antigens of the H-2 system of the mouse could be largely resolved into two allelic series, one at each of two closely linked loci in the K and D regions of the chromosome 17. This observation emphasised the remarkable formal similarity between the principal leukocyte antigen series of man and mouse, and led to the hypothesis that the two allelic series represented products of related genes that had arisen as a result of ancestral duplication. This simplification in turn led to attention being concentrated on the products of these loci—the best defined genetically and serologically in the MHC—as appropriate subjects for biochemical characterisations. In the past few years progressively more detailed analyses of these antigens have led finally, in the past few months, to the first amino acid sequences, the most recent study of which appears in this issue of *Nature*[1].

There is now general agreement that both the H-2D and H-2K products of the mouse, and the HLA-A and HLA-B products of the human MHC are cell surface glycoproteins of total molecular weight approximately 60,000 daltons. They carry about 3,000 daltons of carbohydrate on a heavy chain of approximately 45,000 daltons, and a non-covalently linked light chain of about 12,000 daltons which is known to be β_2-microglobulin. The molecule is inserted into the membrane at the C-terminus, and the whole molecule is soluble only in detergent. A papain fragment of about 36,000 daltons carries all the antigenic activity (as well as the light chain), however, and is water soluble. It is likely that the native molecule exists in the membrane as a monomer, although a free sulphydryl group is available near the C-terminus and may normally participate in some dimerisation. A lymphocyte carries approximately 5×10^5 antigen molecules on its surface.

It has required considerable ingenuity to obtain amino acid sequences from H-2 and HLA antigens because of the minute quantities of material available. By using internal isotopic labelling with individual or small groups of amino acids, four American groups have obtained partial N-terminal sequences of three K end alleles and two D end alleles (Henning *et al.*[2]: Vitetta *et al.*[3]; Silver and Hood[4]; Ewenstein *et al.*[5]). Dissociated immunoprecipitates from detergent-solubilised membrane preparations of normal lymphoid cells were sequenced in the Beckman automatic Sequenator, and the labelled residues identified with appropriate controls. Strominger and colleagues (Terhorst *et al.*[6]) and now Bridgen *et al.*[1] in this issue of *Nature* have obtained partial N-terminal sequences of HLA-A and HLA-B products from lymphoid cell lines. Bridgen and colleagues have adapted solid phase sequencing techniques of whole protein molecules, using a sensitive post-labelling method to identify consecutive residues autoradiographically on two-dimensional thin layer chromatography.

The sequences obtained by these various techniques are summarised in Fig. 1. They demonstrate beyond all dispute the common evolutionary origin of the D and K loci in H-2, and of the A and B loci in HLA. From the substantial numbers of shared residues between H-2 and HLA sequences, a common evolutionary origin for the genes determining both H-2 and HLA is also apparent. The existence of marked species specificity inside the H-2 and HLA sequences seems to

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Mouse H-2	K^b	(Met)	Pro	His		Leu	Arg	Tyr	Phe	Val		Ala	Val		Arg	Pro		Leu		-		Arg	Tyr	Met				Tyr
	K^k	(Met)	Pro	His		(Leu)	Arg	Tyr	(Phe)	His		Ala	Val		Ile	Pro		Leu		Lys	(Pro)	Phe	Ala	Met	His			Tyr
	K^d	(Met)	-	His		-	Arg	Tyr	(Phe)																			
	D^b		(Pro)	-		-	Arg	Tyr	-	-		Ala	Val		Arg	Pro		Leu		-	Pro	Arg	Tyr					
	D^d	Met	(Pro)	His		Leu	Arg	Tyr	Phe	Val		Ala	Val			Pro		-		-	Pro		Tyr					
Human HLA	A2	Gly	Ser		Ser	Met	Arg	Tyr	Phe	Phe	Thr	Ser	Val	Ser	Arg	Pro	Gly		Gly	Glu			Phe	Ile	Ala	Val		Tyr
	B7	Gly	Ser		Ser	Met	Arg	Tyr	Phe	Tyr	Thr	Ser	Val	Ser	Arg	Pro	Gly		Gly	Glu			Phe	Ile		Val		Tyr
	A1,2 }								Phe	Phe	Thr	Ser		Ser														
	B8,13}		Ser		Ser	Met	Arg	Tyr	Tyr	Tyr	Ser	Ala	Val	Ala	Arg	Pro	Gly											

Fig. 1 Collated H-2 and HLA sequences derived from the following sources: H-2, Henning *et al.*[2], Silver and Hood[4], Vitetta *et al.*[3], Silver and Hood[7], Capra, personal communication; HLA, Terhorst *et al.*[6], Bridgen *et al.*[1], Bridgen *et al.* sequenced a mixture of four HLA molecules giving rise to the alternative residue allocations in positions 8, 9, 10, 11 and 13. Residues in parentheses represent either preliminary or disputed results, dashes indicate that the residue present is not any of the other residues known at that position except in position 9 of H-2D where the residue is not valine but may still be histidine. Residues shared between different H-2 sequences are indicated by the vertical blocks, while residues shared between H-2 and HLA are similarly marked in the HLA sequences.

imply that the postulated duplication must have followed the evolutionary divergence of man and mouse, and yet the existence of paired serological loci in many species of mammal suggests the reverse. A resolution of this paradox may be that the genes specifying these antigens show a recurrent tendency to duplicate during evolution. This view might be supported by the existence of a third serological locus, HLA-C, in the human, although the homology of this locus with the A and B loci remains to be demonstrated. The allotypic differences between H-2 specificities at the D and K loci, and between HLA specificities at the A and B loci seem to be reflected in several sequence non-identities between different alleles, for instance at residues 2, 5 and 19 for the three H-2K sequences and residues 3, 5, 8, 9, 17 and 19 for the two H-2D sequences. The frequency of these allotypic substitutions is unprecedented in known allelic systems, and if it is sustained throughout the length of the molecules will pose considerable theoretical problems in accounting for their origin. The conventional models for origin of simple (that is, single substitution) allotypes impose intolerable genetic load on the species when multiple substitutions are to be obtained. Alternative mechanisms to account for the observed differences have been elegantly discussed by Silver and Hood in a recent review (*Contemporary Topics in Molec. Immunol.*, in the press)[7].

It is curious that the sequences reveal no tendency for D products to resemble each other more than they do K sequences, or *vice versa,* since from serological studies the products of the two loci can be shown to differ by locus-specific public antigens. It seems likely however that further sequence data will ultimately confirm the serological results, since in all other respects the implications of serological data have been confirmed by the sequences.

For some time now the notion has been in the air that the remarkable polymorphism in histocompatibility antigens may reflect the specificity of some kind of recognition system, and hence the possibility that H-2 and HLA antigens may show sequence homology with immunoglobulin has been widely canvassed. The association of β_2-microglobulin with these antigens as a "light chain" has reinforced this suggestion in view of the homology of this molecule with an immunoglobulin domain. In the event the sequences of H-2 and HLA antigens so far available do not support the immunoglobulin homology theory, and the absence of a half-cystine anywhere in the first 40 residues also argues strongly against homology. Nevertheless it is possible that sequence homology with immunoglobulin will emerge later on, or alternatively, if the two genetic systems are very distantly related, sequence homology may be absent, but a three-dimensional homology sufficient to account for β_2-microglobulin binding as a light chain homologue may preserved (Hood, personal communication).

There is no doubt that the preliminary sequence data now available for H-2 and HLA antigens have raised as many intriguing problems about the organisation and evolution of these remarkable chromosome regions as they have resolved. It seems certain that as sophisticated microsequencing techniques are further developed there will continue to be a flood of new information about these and other molecules such as Ia or HLA-D in the MHC that will prove as fascinating and intellectually demanding in their interpretation as the immunoglobulin sequences have been.

1 Bridgen, J., *et al., Nature,* 261, 200 (1976).
2 Henning, R., *et al., Proc. natn. Acad. Sci U.S.A ,* 118 (1976).
3 Vitetta, E. S., *et al., Proc. natn Acad Sci U S.A.,* 73, 905 (1976)
4 Silver, J., and Hood, L , *Proc. natn Acad. Sci U.S.A ,* 73, 599 (1976)
5 Ewenstein, B. M., *et al., Proc. natn. Acad. Sci. U S.A ,* 73, 915 (1976)
6 Terhorst, C , *et al , Proc. natn Acad. Sci. U.S.A.,* 73, 910 (1976).
7 Silver, J., and Hood, L., *Cont Topics molec Immunol.* (in the press).

Ineffective nerve terminals

from Shin-Ho Chung

SEVERAL recent studies by Patrick Wall and his colleagues indicate that a class of synapses in the central nervous system is normally inactive but becomes effective if the target cells are deprived of their main inputs. If this notion is correct, it will have an enormous impact in neurobiology. The new concept will not only have a direct bearing on our understanding of how the brain adjusts to injury or altered environment, but may also shed new light on the handling of incoming sensory messages by the brain and the developmental strategies it adopts for forming intricate neuronal networks

For many decades, anatomists and physiologists have explored the projection of sensory afferents onto the mammalian spinal cord Together they have slowly compiled a story of almost incredible complexity. Cutaneous fibres originating from a circumscribed region of the skin (a dermatome) bundle together to form a dorsal root and enter the cord at the appropriate spinal segment. Once these fibres enter the cord, they branch out explosively to synapse with thousands of neurones in the grey matter. A fibre initially divides into a T-shaped junction, and the two main stems, traversing several segments in the rostral and caudal directions, drop their numerous collaterals onto the cell layers beneath. It has been estimated that major collaterals are given off from the stems at intervals of 100 to 200 μm. Since the radiation of sensory fibres continues over a region covering many segments, several hundred, major collaterals can stem from any one fibre.

In apparent contradiction to these complicated, diffuse patterns of interconnections between sensory afferents and spinal neurones, physiologists have revealed a strict somatotopic map of the skin in the spinal cord. Each dermatome is represented by one or at most two segments of the spinal cord. Cells located medially in the dorsal horn synapse with fibres representing the distal part of the dermatome, whereas those located more laterally represent the proximal part of it. For example, each large cell in lamina IV (to which the large myelinated fibres carrying cutaneous information project) subserves a small patch of the skin, usually less than the size of one toe, and gentle pressure or movement of hair within this receptive field causes a vigorous discharge on that cell (Wall, *J. Neurophysiol.*, **23,** 197; 1960; Brown and Fuchs, *J. Neurophysiol.*, **38,** 1; 1975). Stimuli applied to areas outside this skin region fail to show any effect on the cell, despite the anatomical evidence for a widespread dissemination of sensory information within the cord.

This discrepancy between anatomical and physiological descriptions of the spinal cord has been a persistent worry to Wall. For if a single fibre spreads its terminals over 10 spinal segments, it is not immediately apparent how cells in one segment can have a restricted dermatome and maintain a strict somatotopic order within themselves. Wall and Werman (*J. Physiol., Lond ,* **255,** 321; 1976) have recently re-examined the central projection of spinal afferents and have confirmed the earlier anatomical descriptions. Using the technique of antidromic stimulation, they have traced the trajectory of single axons in the spinal cord of the cat. Some myelinated fibres invading the cord at Lumbar 2 segment, for example, have their terminals extended as far caudally as Sacral 1, which is 6 segments away from their zone of entry. Stated conversely, cells in a given segment receive not only fibres from their nearest dorsal root but also distant arborisations radiating from many neighbouring dorsal root fibres.

According to Wall (*Phil. Trans. R. Soc. Lond.,* in the press), the synapses formed by distant branches normally remain dormant, but become active as a result of changes triggered by de-afferentation. This assertion is based on several related observations on lamina IV cells after cutting the appropriate dorsal roots. All fibres which form effective synapses with the cells are contained in the dorsal root nearest to them; if this root is cut, the cells become unresponsive to tactile stimulation, and the dermatome which is subserved by it becomes insensate. Merrill and Wall (*J Physiol., Lond.*, **226**, 825; 1972) first discovered that within a few hours after surgery, de-afferented cells begin to respond to electrical stimulation delivered to neighbouring dorsal roots, although natural stimulation anywhere on the skin cannot excite them. One week after de-afferentation, the cells can be excited by light brushing, touch or presure on regions of the skin which they normally would not represent (Basbaum and Wall, *Brain Res.*, in the press). Some of these cells are connected to two disparate parts of the body, for example, abdomen and leg. Hints of a similar phenomenon exist elsewhere in the mammalian brain. Cells in the thalamic nucleus of the rat (Wall and Egger, *Nature*, **232**, 542; 1971) and in the gracile nucleus of the cat (Millar *et al.*, *Expl Neurol.*, **50**, 658; 1976) change their receptive fields from leg to arm or trunk when the pathways connecting the leg to these nuclei are destroyed. These findings, taken together, do indeed suggest that axons in the brain form many more synaptic contacts than are apparently needed in normal circumstances. It should be noted, however, that various other interpretations, of which sprouting of neuronal terminals into vacated territory is one, have not been conclusively eliminated.

There are several possible ways in which a synaptic terminal could be rendered ineffective. Nerve impulses travelling down an axon may invade only a few proximal branches and fail to invade the remaining terminals of the axon. Alternatively, the distant terminals may terminate at distant segments of the dendrites of their target cells, thereby making the contacts less effective in bringing the target cells to the threshold of firing. Finally, the distant terminals may terminate on their target cells not directly but through small interneurones. In any of these cases, changed conditions in the tissue induced by de-afferentation could conceivably cause the presence of such distant terminals to be more apparent.

Whatever the mechanism ultimately turns out to be, Wall's findings pose the intriguing possibility that during development a growing axon forms diffuse contacts with a large number of target cells, and through use and disuse some of these contacts remain effective whereas the others become ineffective. The ineffective terminals would remain, in Merrill and Wall's phrase, as "ghosts of the cell's childhood" and become functional again if the neuronal circuitry needed to be modified. This line of speculation leads to testable predictions about early developmental processes of somatotopic organisation. For example, a lamina IV cell in a young kitten could be excited by sensory fibres stemming from disparate regions of the skin, and ordered representation of cutaneous receptive fields onto spinal neurones could evolve gradually as the animal matures. Now and for the next few years, as more direct approaches are used to reveal the mechanisms governing development and plasticity of the nervous system, the observations of Wall, and the interpretations he has drawn from them, will remain as a valuable guide. ☐

Brightness variations of Saturn's Rings

from David W. Hughes

ASTRONOMERS, since 1921, have occasionally reported that the brightness of Saturn's rings varies with azimuthal angle (the angle θ in Fig. 1). The degree of variation is a matter of considerable debate, however, and papers have been published claiming that the eastern ansa of the inner ring B is brighter than the western one, fainter than the western one, and that each ansa is fainter than the ring position directly in front of Saturn (that is $\theta=90°$). The outer ring A is also found to vary in brightness, but in a more complicated way.

Hoping to solve this ambiguity Karl Lumme and William Irvine of the Department of Physics and Astronomy, University of Massachusetts, have made careful photometric studies of some 100 good-quality Saturn plates from the New Mexico State University and Lowell Observatory collections. The preliminary results are reported in a recent edition of the *Astrophysical Journal* (**204**, L55; 1976). The brightness of each ring as a function of θ has been examined for each of six wavelengths in the band 3,600 to 8,000 Å and also for phase angles, α, (the angle Earth–Saturn–Sun) larger or smaller than 1° and for ring tilt angles, B, larger or smaller than 20°. Results show that at 5,500 Å, $\alpha > 1°$ and $B > 20°$, the outer ring A has a maximum of brightness at $\theta=45°$ and 225° and a minimum (about 10% lower) at $\theta=135°$ and 315°. The effects seem to be less pronounced at shorter wavelengths.

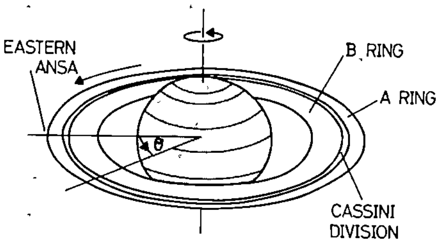

Fig. 1 Saturn and its rings. The brightness of the A ring varies with θ, the azimuthal angle, whereas the B ring seems to have a constant brightness.

For the inner B ring Lumme and Irvine find small maxima in brightness for $\theta=0°$ and 180°. This is caused by the image spreading on the photographic plate due to the finite resolving power of the telescope, atmospheric effects and photographic irradiation on the plate. When corrections are made for this spreading, the resulting brightness is found to be independent of θ.

The authors conclude from their results that the particles in ring A may have rotation periods locked to their period of revolution about Saturn. So just like the Moon they always point the same face to the central planet. The above data also indicate for the first time that the average properties of the particles in ring A may differ from those in ring B.

Morrison (*Icarus*, **22**, 57; 1974), who studied the rings by infrared radiometry, found that the particles at the western ansa had a higher brightness temperature than those at the eastern ansa. He proposed that the particles have uneven albedoes, the brighter sides being orientated towards their direction of orbital motion.

The nature and size of the particles in the rings are still poorly understood. Pollack (*Space Sci. Rev.* **18**, 3; 1975) reviewed recent visual, infrared, radio and radar data and concluded tentatively that the mean particle size is around 10 cm, the bulk composition being, in the main, water ice. These particles occupy between 0.5 and 1% of the total volume of the rings. The physical thickness of the rings is about 100 m but the rings do bulge slightly at some of the period resonances with satellites Mimas and Titan. The total mass of material in the rings is small, Pollack's figures leading to a value in the region of 10^{18} g, which is about the mass of a large comet and is over a million times less massive than the Moon.

It is hoped that this picture will be greatly clarified in the next decade when the data from the Pioneer and Mariner Jupiter/Saturn space probes have been received and analysed. ☐

Sexual selection and the handicap principle

from John Krebs

DARWIN developed his theory of sexual selection to account for the extravagant secondary sex characters possessed by males of many species. Peacock tails, deer antlers, and bird of paradise plumes are classic examples of characters which hamper survival, but which may increase mating success. Darwin's theory was neatly formalised by R A. Fisher 36 years ago in his book *The Genetical Theory of Natural Selection* and later developed as a genetic model by O'Donald (*Heredity*, **17**, 541; 1962); briefly, the argument goes like this. Females on the whole tend to be fussier in their choice of mates than are males (the female has more to loose if she makes a bad choice because eggs are more expensive than sperm), so that males end up vying with one another for the attention of a female. Females will always try to choose partners of 'good quality'—those which make good parents, or which have good genes to pass on to their children—and in the course of evolution females will develop a preference for external indicators of these qualities. Now suppose that females have begun to evolve a liking for males with green heads, an indicator of good quality: immediately a new selective force comes into play. Males with green heads will now be at an advantage because they are popular with females, and therefore females which choose green-headed males will have sexually attractive sons (assuming inheritance of the green colour), regardless of the quality of these sons in any other respect. The female preference itself generates a selective advantage for green heads, which in turn favours a stronger female preference. It is this positive feedback loop which has led to the extraordinary elaboration of many male sex characters

Recently, Zahavi (*J theor. Biol.*, **53**, 205–214; 1975) has come up with an audacious alternative theory of sexual selection. He argues that the females' taste for males with costly, survival-reducing adornments evolves precisely because these features are a handicap. A male which has survived to breeding age in spite of dragging around a huge tail since birth has overtly demonstrated his superior fitness, just as if two athletes ran a mile in four minutes, but one carried a sack of coal, we would conclude that this individual was stronger than his rival. The handicap principle, as Zahavi calls it, has an immediate intuitive appeal. A female benefits by preferring the most handicapped male, because he will pass on good genes to her children, and the handicapped male benefits because the heavy cost of his adornments prevents easy copying by other males. Unfortunately, however, the handicap principle does not work. Maynard Smith (*J theor. Biol.*, **57**, 345–354; 1976) and Davis and O'Donald (*J. theor. Biol.*, **57**, 239–242; 1976) have both analysed Zahavi's idea in more detail, and they show (as many people had suspected) that the verbal argument has serious flaws when translated into strict genetic terms.

Both critiques point to an obvious problem. Even if a handicapped male passes on good genes to his children, he unfortunately also passes on his handicap, which must, according to Zahavi's hypothesis, reduce their survival chances. Perhaps the idea can be salvaged by assuming that the good genes are passed to male and female offspring, but the handicap only goes to the male? Davis and O'Donald point to two difficulties with this argument. First, it is unlikely that genes which are good for a highly adorned male will also be optimal for a cryptic female, and second even if one assumed that the same genes were optimal for both sexes, selection would soon bring females to near their optimum genotype, at which point the advantage of choosing a handicapped male would fade away.

Maynard Smith puts Zahavi's verbal argument into the form of a simulation model, using three loci, one for fitness (high and low), one for a handicap such as tail length, and one for female choosiness—the choosy allele only mates with handicapped males. The fitness of a 'high fitness–no handicap' male is set at 1, and the other fitnesses are calculated by subtracting a cost of the long tail, and a cost of the 'low fitness' allele. In order to eliminate the effect of female preference (Fisher's idea) the simulation was started with a very low frequency of the 'choosy' allele, the other loci being represented by equal frequencies of their two alleles. The results of the simulation were unambiguous: choosy females and handicapped males declined in frequency, and this was true for a wide range of fitness values. The handicap itself does not favour the evolution of elaborate male sex displays.

Interferon deglycosylated

from David Metz

INTERFERON is the cellular protein, synthesised by virus-infected animal cells, which effects the protection of other as yet uninfected cells against subsequent virus infection. Interferon has long been believed to be a glycoprotein. The carbohydrate substitution is to be expected of a secreted protein though the experimental evidence for its occurrence has hitherto been somewhat indirect. The principal impediment to the full biochemical characterisation of the various molecular species of interferon has been the difficulty of purifying the material to demonstrable homogeneity. The reason for this difficulty lies in the very high specific activity of interferon which means that large volumes of crude preparations (necessarily from tissue culture) yield tiny amounts of pure interferon.

Progress in purifying interferon has been steady if unspectacular in recent years. The current state of the art is well illustrated by two recent papers from Knight on the purification and characterisation of mouse and human interferons. Using basically similar purification procedures, both mouse L cell interferon and human diploid fibroblast interferon were purified to specific activities of 2×10^8 units per mg protein. The human interferon preparation, when characterised by polyacrylamide gel electrophoresis, displayed a single peak containing essentially all the interferon activity, protein staining capacity and carbohydrate staining capacity of the preparation (Knight, *Proc. natn Acad. Sci. U.S.A.*, **73**, 520; 1976). This preparation, containing a single polypeptide of apparent molecular weight 20,000, would seem to be the first homogeneous preparation of human interferon.

In contrast, the mouse interferon yielded some dozen discrete bands of activity, following polyacrylamide gel electrophoresis, spanning an apparent molecular weight range of 20,000 to 32,000. About half of these components were demonstrated to contain carbohydrates, as judged by the periodic acid-Schiff base staining procedure (Knight, *J. biol. Chem.*, **250**, 4139; 1975). The reason for this heterogeneity of mouse interferon is as yet undetermined. Variation in the carbohydrate content, as opposed to the primary amino acid sequence, is clearly one possibility.

A second type of heterogeneity in which a variable degree of glycosylation appears to be implicated is the charge heterogeneity of interferon which may be examined by isoelectric focusing. Dorner *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1981; 1973), confirming the earlier work of Schonne, Billiau and De Somer, demonstrated that treatment of rabbit interferon with neuraminidase converted the native heterodisperse charge distribution to a single component with no loss of biological activity. Since neuraminidase would be expected to cleave a terminal sialic acid residue from a penultimate galactose in a carbohydrate side chain it was inferred that native rabbit interferon contained variable amounts of sialic acid as the terminal sugar which, moreover, was not involved in the antiviral function of the molecule.

This type of investigation has recently been extended to human interferon by Anfinsen and his colleagues (Bose *et al.*, *J. biol. Chem.*, **251**, 1659; 1976) who have shown that 80% of the sugar moieties may be removed from a partially purified interferon preparation using a mixture of glycosidases, again without any loss of biological activity. Unfortunately, since the interferon preparation used was of undefined purity one cannot estimate how much of the carbohydrate of the interferon molecule itself was removed in this experiment. Nevertheless, the demonstration of both size and charge changes of the interferon activity following enzymic digestion do support the notion that at least some of the sugar residues may be removed from human interferon without loss of biological activity.

If it turns out that all the carbohydrate is dispensable without loss of activity the eventual chemical synthesis of interferon would be facilitated. But whether such a synthetic molecule free of carbohydrate side chains would be therapeutically useful is uncertain. One general feature of studies in which interferon has been used to attempt the protection of man and animals against virus challenge has been the relatively large quantities of interferon required for efficacy This may well be a consequence of the common finding that interferon has a short half-life in the circulation. It is known that a variety of glycoproteins are rapidly cleared from the circulation when even a limited number of the normal terminal sialic acid residues are removed (Ashwell and Morell, *Advances in Enzymology*, **41**, 99; 1974). Thus Dorner *et al.*, (*op. cit.*) have suggested that the half-life, and consequently the therapeutic usefulness, of interferon might be enhanced if the native heterogeneity were diminished by the additional incorporation of sialic acid residues. So reglycosylation of synthetic or natural interferons might in fact enhance their therapeutic value. ☐

Cross section for nuclear fusion

from P. E. Hodgson

When two nuclei collide, there is a certain probability, depending on the energy and on the nuclei, that they will fuse to form a compound nucleus, which subsequently decays by the emission of neutrons, charged particles and gamma rays. In recent years there have been many studies of the cross section for nuclear fusion, and some features of the process are now understood (*Nature*, **256**, 261; 1975).

The probability that two nuclei fuse depends on the impact parameter, the shortest distance between their initial directions of motion. If the collision is a glancing one (large impact parameter), a few nucleons may be transferred from one nucleus to the other, or knocked out entirely, but the nuclei will go essentially unchanged. If the collision is more or less head-on (small impact parameter), the nuclei will interact strongly and are likely to fuse.

The probability of fusion also depends on the energy of the collision: at low energies the nuclei are kept apart by the electrostatic repulsion and no reactions can occur. As the energy increases, so does the fusion probability, until at very high energies it begins to decrease because the nuclei are more likely to be shattered by the violence of the impact.

These collisions are far too complicated to be treated in detail by quantum mechanics, but substantial progress has been made towards understanding the fusion cross section by using classical ideas (*Nature*, **256**, 261; 1975). If R is the impact parameter, then the angular momentum of the lighter ion about the heaver is

$$L\hbar=mvR=k\hbar R\ .$$

If all collisions with impact parameters less than R lead to fusion, then the total fusion cross section

$$\sigma_f=\pi R^2=\pi^2L^2/k^2$$

As the energy increases, the peripheral collisions bring in more angular momentum than the compound nucleus can accept, so fusion can only occur for angular momenta less than some limiting value $L_F<L$ and then

$$\sigma_F=\pi^2L_F^2/k^2$$

Very many experimental studies of the fusion of nuclei have now been made, and it is found that the fusion cross sections behave in a systematic way. In particular, measurements have been made of the fusion cross section for several different pairs of nuclei giving the same compound nucleus at the same energy, and also of the same reaction at a series of energies. These have shown that fusion is not a static phenomenon; it depends on the dynamics of the interaction process.

Conditions required

A careful analysis of a wide range of fusion cross sections by Galin and colleagues (*Phys. Rev.*, **C9**, 1018; 1974) led to the remarkably simple conclusion that all the data could be accounted for by assuming that fusion takes place as soon as the interacting nuclei come closer than a distance 1.1 $(A_1^{\frac{1}{3}}+A_2^{\frac{1}{3}})$ fm where A_1 and A_2 are the atomic weights of the two ions. A similar analysis of data on other nuclei was made by Gutbrod and colleagues (*Nucl. Phys.*, **A213**, 267; 1973) and they came to a similar conclusion, but with the difference that the distance that fitted their data was 1.4 $(A_1^{\frac{1}{3}}+A_2^{\frac{1}{3}})$ fm. These two results are significantly different and it is important that they be reconciled.

A theory that does this has been developed by Glas and Mosel (*Nucl. Phys.*, **237**, 429; 1975). It provides a simple model of the fusion process by assuming that the potential in the region of interest has a parabolic form with depth $V_B+\hbar^2L(L+1)/2\mathscr{J}_B$ where L is the orbital angular momentum and $\mathscr{J}_B$ the nuclear moment of inertia. This enables the fusion cross section to be calculated analytically, and the formula obtained fits all the experimental values. At low and high energies, it gives to a good approximation the values found by Gutbrod *et al.* and Galin *et al.*, and thus unifies them coherently.

The reason for the change of slope is connected with the upper limit to the relative angular momentum of the two nuclei for which fusion can occur. This upper limit is set in two ways, and since both limits must be obeyed it is the lower of the two that actually

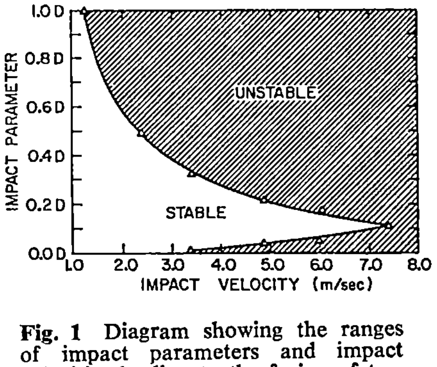

Fig. 1 Diagram showing the ranges of impact parameters and impact velocities leading to the fusion of two equal drops of water (unshaded). The impact parameters are expressed as fractions of the drop diameter $D=300\ \mu\text{m}$. The power limit to fusion at $3.4\ \text{m s}^{-1}$ has a counterpart in nuclear collisions.

determines the fusion cross section. The first limit is set by the nuclear radius R; the angular momentum cannot exceed mvR, where m and v are the mass and velocity of the lighter of the two ions. In practice glancing collisions do not lead to fusion so the limit is somewhat less than this and allowance must also be made for the reduction of the velocity of the incident nucleus by the Coulomb repulsion.

The other limit is set by the maximum angular momentum that can be given to the nucleus without causing it to fly apart. This is fixed by the internal structure of the nucleus and can be estimated by the liquid drop model. At low energies this limit is not important, and the fusion cross section is limited by the nuclear radius. As the energy increases, the angular momentum mvR increases until it reaches the critical value set by the limit of nuclear stability, and thereafter the fusion cross section is limited by the latter criterion.

These two limits correspond to the two different formulae for the separation between the nuclei for fusion to take place, and the transition region to the value of the angular momentum for which the two limits are approximately the same.

The theory of Glas and Mosel thus very neatly accounts for the variation with energy and with interacting nuclei of all the fusion cross sections available when they completed their work. Since then, however, further results have become available that cannot be accounted for by their theory, at least in its original form. The reason seems to be quite well understood in one case, but not in the other.

The first set of anomalous results was obtained by Gauvin and collaborators (*Phys. Rev.*, **C10**, 722; 1974). In the collision of two quite heavy nuclei, ^{74}Ge and ^{84}Kr, fusion takes place at a higher energy than is expected from the results for the collision of ^{40}A with ^{118}Sn, which leads to the same compound nucleus. This energy shift is as high as 15 MeV and was totally unexpected and quite outside the uncertainties of the experiment.

A possible explanation of this anomaly is that there is a lower as well as an upper limit to the impact parameter for fusion. It is then easy to adjust the lower limit to give the observed threshold shift, but then one wants to understand why fusion cannot take place for low impact parameters and how this varies with energy and from one nucleus to another.

Liquid drop model

A way of doing this has recently been suggested by Natowitz and Namboodiri (*Phys. Rev.*, **C12**, 1678: 1975), making use of the analogy with the collision of two liquid drops. This has been studied by Adam and colleagues (*J. appl. Phys.*, **39**, 5173; 1968), and they did indeed find that at some energies there is a lower as well as an upper limit to the impact parameter for the fusion of the two drops.

Some of their results are shown in Fig 1, which gives the impact parameters (expressed as fractions of the drop diameter D) for fusion as a function of the impact velocity in m s^{-1}. This diagram is easily understood: fusion takes place for low velocities and impact parameters but if either or both are large the drops either brush past each other or shatter.

The most interesting region occurs for low impact parameters and impact velocities greater than 3.4 m s^{-1}: even though the impact parameter is small fusion cannot take place, and this is just the effect that we want to explain in the collisions of heavy nuclei.

This effect can be understood by considering the collision in more detail. When fusion takes place, the kinetic energy of the colliding drops is transformed into the rotational and vibrational energy of the combined system. Now the kinetic energy can only go into rotational energy if the impact parameter is greater than zero, and the higher the impact parameter the more rotational energy can be given to the compound system.

For the extreme case of a head-on collision (zero impact parameter) the incident energy can be entirely transformed into vibrational energy for low impact velocities, but at the critical velocity of 3.4 m s^{-1} this is no longer possible. This corresponds to the maximum energy that can go into vibrations of the compound system without breaking it up.

If now the impact parameter is increased, some of the energy can go into rotational energy, so fusion can again take place. Thus for impact velocities above the critical value there is a lower limit to the impact parameter for fusion; below this impact parameter the system cannot be given enough rotational energy to absorb the energy remaining from the incident kinetic energy after as much as possible has been put into vibrations. At still higher impact parameters the rotational energy is so large that the two nuclei cannot stick together and fusion again cannot take place. There is thus a limited range of impact parameters for which fusion can occur.

As the energy increases, so does the rotational energy for a particular impact parameter, so that the lower limit to the impact parameter for fusion rises and the upper limit falls. Eventually at 7.4 m s^{-1} they coincide, and for higher velocities fusion cannot take place at all.

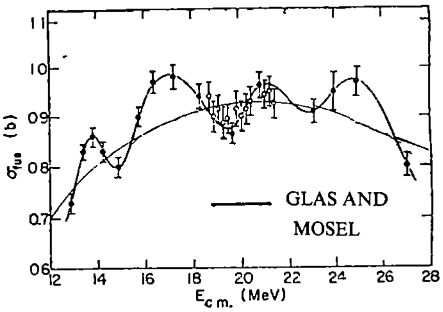

Fig. 2 Fusion cross section for the interaction of ^{12}C and ^{16}O showing the unexplained oscillatory structure superposed on the dependence given by the theory of Glas and Mosel.

The lower limit to the impact parameter for nuclear fusion can thus easily be understood by considering the behaviour of liquid drops. One important difference is the charge on the nuclei which prevents fusion at smaller impact velocities. For light nuclei the fusion threshold is below the critical impact velocity and so all impact parameters are allowed. The earlier experiments were all in this region and it was here that the systematic behaviour already mentioned was established.

For heavier nuclei, however, the fusion threshold is above the critical impact velocity and so only a limited range of impact parameters leads to fusion. There is thus an energy shift in the curve of fusion cross section as a function of energy compared with the curve that would have been obtained if the lower impact parameters also led to fusion. This higher fusion threshold is just what is observed experimentally for collisions between heavy nuclei.

The second set of data that cannot be understood by the theory of Glas and Mosel is the fusion cross sections of the interaction ^{12}C+^{16}O from 13 to 27 MeV measured by Sperr and colleagues. (*Phys. Rev. Lett.*, **36**, 405; 1976). As shown in Fig 2, the experimental fusion cross sections oscillate around the mean value given by the theory of Glas and Mosel, and it is not at all clear how this behaviour can be explained. One possible explanation, that it is due to the effect of successive partial waves as they come in turn to dominate the reaction, is ruled out because it gives oscillations that have too small a period and too small an amplitude.

Other explanations are that it is due to the effect of resonances in other channels, or to a resonant transfer process (*Nature*, **246**, 14; 1973) According to this explanation, a nucleon can be transferred back and forth between the two approaching nuclei, and this could produce a periodic modulation of the fusion cross sections. This mechanism gives a very characteristic variation of the fusion cross section with energy, and this is approximately in accord with the experimental data. □

review article

Short term screening tests for carcinogens

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There are now short term tests with a high predictive value for mammalian carcinogens. Many of them are based on the ability to detect damage to DNA in bacteria or mammalian cells after metabolic activation by microsomal enzymes. Their introduction will enable provisional safety assessments to be made for the many thousands of industrial and environmental chemicals for which long-term animal testing cannot at present be considered.

It has been estimated[1] that if one could totally abolish human cancer it would add a mere two years to the average lifespan. Most cancer sufferers are past retiring age so that industrial production would be little affected by the abolition of cancer. The fight against cancer must instead be justified in terms of the cost of hospital services and of basic humanity; treatment of cancer, even when it is successful, is a miserable process. When it fails, as it so often does, one feels guilty of a double offence, not only the loss of the patient, but the imposition of heroic measures that themselves may cause considerable physical and mental suffering.

The International Agency for Research on Cancer holds it as a rule of thumb that around 80% of cancer has an environmental cause[2,3]; others would give a higher figure[4]. The evidence is indirect, being based on differences in tumour incidence between genetically similar populations in different environments[2,3,5]. Even if this estimate is only approximately correct it leads ineluctably to the conclusion that a substantial proportion of cancers, possibly a majority, are in principle preventable. In past decades those responsible for the disbursement of cancer research funds have tended either to look for a breakthrough in the area of curative treatments or to make a long term investment in basic biology in an attempt to understand the disease (or more properly diseases since "cancer" is but a general term for hundreds of different malignant conditions). Recently, however, these two essential approaches have been complemented by a third, the search for the specific environmental factors involved in carcinogenesis.

The nature of these environmental factors is not known in detail, but it seems likely that many of them are man-made or natural chemicals. Even factors such as diet or stress may act indirectly by altering the metabolism of chemicals in the gut or in the body itself. Of course, identification of environmental carcinogens does not necessarily lead to their removal but it does open the way to control so that the risk that they present is no more than is necessary when weighed against any benefits that they may give.

The most direct method of identifying environmental carcinogens for man is based on population studies, but unfortunately it is expensive and seems to have rather low resolving power. Only a handful of chemicals are known to be carcinogenic to man and most of these have been detected following the study of workers occupationally exposed to chemicals capable of giving rise to specific and rather rare neoplasms. The classic case is soot which has been known for 200 years to produce scrotal cancer in young chimney sweeps[6]. More recent examples are 2-naphthylamine, vinyl chloride and asbestos which produce, respectively, rare cancers of the bladder, angiosarcomas of the liver, and mesotheliomas of the lung cavity. The problems involved in identifying two populations differing only in their exposure to one chemical are formidable and are further compounded if the chemical gives rise not to specific and otherwise rare tumours, but to a variety of common cancers. Population studies are thus likely to be of limited value in identifying environmental (as distinct from occupational) carcinogens but they will be indispensable in providing the basis for risk evaluation, particularly where dose–response data can be obtained.

The alternative is to screen chemicals to which man is exposed. The generally accepted method of doing this is to carry out long term carcinogenicity tests with laboratory mammals. Not only are those tests very demanding of resources but any extension of animal testing on such a wide scale would be vigorously opposed by a number of animal welfare lobbies. In practice, it is inconceivable that resources could be made available (either men, money or mice) on the necessary scale to screen all the tens of thousands of substances to which humans are exposed. Of necessity, therefore, testing with whole mammals will be restricted to certain groups of suspect substances, for example those suspect but already in use on a large scale, or those substances which it is proposed to administer on a large scale, as food additives or cosmetics, for instance.

If one is to screen for carcinogenic chemicals, therefore, one must use short term tests with a high predictive value. I propose to review a number of possible systems which have been suggested in recent years. As will become apparent, many of them are in fact systems for the detection of agents causing damage to DNA. Damage to DNA leading to heritable changes may be important to man not only because of carcinogenicity but because it may cause hereditary disease[7–9]. Moreover, DNA damage may conceivably be involved in ageing and diseases associated with ageing[1]. I take it as self-evident that any agent likely to damage the DNA of man, whether in somatic or germ cells, is potentially hazardous.

Screening systems

The induction of cancer is but one aspect of long term toxicity and for the evaluation of such hazards a three-tier

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approach has been proposed[10,11] The first tier would consist of simple short term sub-mammalian tests with a high predictive value for the human effect ultimately of interest. As many substances as possible should be screened with these tests. Second-tier tests would be both short and long term, on mammals. Only selected, high priority substances would be screened by these tests in addition to the first-tier tests. Tests in the third tier are designed not to detect toxic agents but to evaluate as quantitatively as possible the hazards to man from agents shown to be potentially toxic. Only substances whose use or presence seems inescapable would be subject to the third tier of evaluation, the object of which would be to make a risk–benefit assessment and institute appropriate regulatory action. Tests in successive tiers show in principle increasing relevance to man but this is often accompanied by decreasing sensitivity and practicability.

Not all of the sub-mammalian tests depend upon the postulated electrophilic nature of the active forms of carcinogens and in particular on their ability to react with DNA. Williams and Rabin[12], for example, have proposed that substances might be screened using a test based on membrane–polysome association. They found that a number of carcinogens caused degranulation of rough endoplasmic reticulum (microsomal membranes) from male rat liver. This test has been further developed by Purchase and Lefèvre[13] who have measured the loss of radioactive RNA from rough endoplasmic reticulum. Preliminary results (D Anderson *et al.*, unpublished) with a large number of carcinogens and non-carcinogens indicate that the method predicts the activity of arylamines rather well (85% correct) although it is less successful with polycyclic hydrocarbons and direct acting alkylating agents.

The necessity for metabolic activation of many carcinogens by microsomal enzymes prompted the suggestion of McPherson *et al.*[14] that the specific *in vitro* enhancement of biphenyl 2-hydroxylation activity in rat liver microsome preparation might be used as a screening test. They found that of eight known carcinogens, all caused an increase of around 100% in such activity; four compounds whose carcinogenicity is in doubt gave lower but significant increases, and eleven non-carcinogenic compounds gave no significant increase. This test system, like that of degranulation of ribosomes, is obviously promising and in need of a much more exhaustive validation on a scale similar to that used with some other systems.

Metabolic activation

There is a widespread belief among cancer workers that DNA damage is involved in the induction of cancer. That is the basis for the supposition that carcinogens might be detected by the consequences of DNA damage in simple systems. Two recent developments have enabled this possibility to be realised. First, it has become clear that many carcinogens are the products of metabolism of inactive chemicals by mixed function oxidases in the animal[15], and that preparations of liver microsomes can be used *in vitro* to carry out this metabolic activation[16–18]. Second, ultra-sensitive bacterial systems, usually involving strains deficient in DNA repair, have been developed for the detection and characterisation of agents causing damage to DNA[19–23].

The first published work in which the mutagenic activity of metabolites was detected after metabolic activation of carcinogens was by Malling[17]. The methodology of his quantitative liquid assay system has been recently described[24]. Later, Ames *et al.*[25] showed that microsomes could be added to the semi-solid agar overlay in a plate test, a procedure that is in some ways rather better for routine screening although it fails with a few compounds, for example, dimethylnitrosamine, possibly because the agar interferes with the diffusion of short-lived active metabolites.

Bacteria deficient in repair of DNA are killed more easily by DNA-damaging agents than are wild type bacteria, and this is the basis for several simple tests. Bacteria deficient in excision repair have been used[18] but these are sensitive only to certain types of DNA damage. Much more useful have been bacteria lacking DNA polymerase I (Pol$^-$)[26], or deficient in genetic recombination (Rec$^-$)[27]. These tests are usually conducted on the surface of agar plates but are also amenable to rather more quantitative procedures with liquid-phase treatment[28,29].

Another way of revealing the existence of DNA damage is to look for the repair that it usually initiates and this is the basis of a very useful test developed by Stich and his colleagues. It depends on estimating the amount of DNA synthesis involved in repair by measuring autoradiographically the uptake of tritiated thymine during the period immediately following exposure to the test chemical. The method has the advantage that it can be used with cultured human skin fibroblasts. To prevent normal DNA synthesis the cells are kept in an arginine deficient medium for 3 d before exposure.

In a report on 64 substances tested, Han and Stich[30] found that all directly acting carcinogens elicited unscheduled DNA synthesis whereas no repair synthesis was observed after treatment with 16 non-carcinogens. Most carcinogens known to need metabolic activation gave negative results although a few were active after prolonged exposure to high concentrations. More recent results[31] indicate that metabolic activation systems can be incorporated in this assay and make possible the detection of procarcinogens.

Mutation induction

Perhaps the most sensitive assay for DNA damage is the induction of mutations in bacteria, particularly if the bacterial strain carries a mutation rendering it unable to excise damage from DNA (Uvr$^-$). Excision-proficient strains should always be included in any assay, however, because certain agents able to cross-link DNA are only mutagenic in such strains[32]; presumably the mutational event occurs as an error during excision-initiated repair. Reversion to prototrophy is generally regarded as the most sensitive type of assay and the methodology has recently been reviewed[33,34]. *Escherichia coli* WP2 is a tryptophan-requiring strain that responds to mutagens causing base-pair substitution mutations at both adenine : thymine and guanine : cytosine sites.

A more complete set of tester strains has been developed in *Salmonella typhimurium* by Ames and collaborators[21]. Individual strains respond to base-pair substitution mutagens or to compounds causing various types of frameshift. The permeability of these *Salmonella* strains to some chemicals has been increased by the incorporation of a cell wall mutation ("deep rough")[22]. From recent data one can calculate that these strains are capable of detecting mutagenic activity of between 61%[35] and 90% (D. Anderson, unpublished) of known carcinogens. In an attempt to detect the "false negatives" obtained with the deep rough strains, Ames's group developed a fourth generation set of strains containing the drug resistance plasmid pKM101[23]. As suggested by MacPhee certain plasmids confer a mutator activity on their host cell which becomes more sensitive to many mutagens and carcinogens[36]. The ability of these plasmid-containing strains to detect carcinogens as mutagens is impressive (see below). A word of caution is in order, however, since although the mechanism by which the plasmids act is still unknown, it is clear that they convert into mutations damage which would not be mutagenic in a normal cell. They may even act as amplifying systems and produce mutations at sites where no damage exists. The value of such strains lies in the correlation they show with carcinogenicity but there is at least a theoretical possibility of real "false positives".

It is possible to improve sensitivity by altering the methodology, and a modified fluctuation test has been proposed which achieves between ten and one hundredfold greater sensitivity than the conventional assay without the need for plasmid-containing strains[37]. There is also the possibility of developing a single tester strain that can be used to detect many different types of mutational events[38]. Although bacterial screening systems have proved very useful, there is still scope for further improvement.

As well as the bacterial tests that have now been extensively studied, a large number of other techniques can be used to detect DNA damaging activity and may perform a useful supplementary role, probing ambiguous or suspect results and characterising more fully the nature of the genetic damage. One may, for example, study the induction of mutations in cultured mammalian cells[39–41]. Mammalian cells may also be used for cytogenetic study of visible chromosome aberrations[42]. Recently developed staining techniques for demonstrating sister-chromatid exchanges show a greatly enhanced sensitivity[43] and their role in screening has been recently discussed[44]. Sister-chromatid exchanges may now be detected in spermatogonia[45] and bone marrow cells[46] following exposure of the whole animal to carcinogens. It is already clear, however, that although the induction of sister-chromatid exchanges is a very sensitive response to some carcinogens it occurs hardly at all with others[43]. Other eukaryotic mutation systems include fungi, yeasts and insects. All of these have their own advantages and disadvantages.

Malignant transformation in cultured cells

Rather than develop a model system depending on mutation or DNA repair, others have worked towards a screening method by which transformation to the malignant condition could be brought about and detected in cell culture. The only really valid criterion for malignant transformation is the ability of a cell to produce a tumour when inoculated into an appropriate host. There are, nevertheless, several secondary criteria (discussed by Freeman and Huebner[47], of which the most commonly used is the ability of cells to grow into clones in soft agar or, in the case of fibroblast cultures, to produce clones of piled-up cells when growing on a solid surface.

Most human cancers are carcinomas which are derived from epithelial cells Relatively little work has, however, been done on the transformation *in vitro* of epithelial cells. Such cells are usually obtained from rat liver and are not easy to retain in culture in the differentiated state. Nevertheless they have been successfully transformed by 4-nitroquinoline-1-oxide[48], aflatoxin B_1, *N*-hydroxy-2-acetylaminofluorene, and 7,12-dimethylbenz[*a*]anthracene[49], dimethylnitrosamine and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine[50], and *N*-acetoxy-2-acetylaminofluorene[51]. Epithelial cells transformed *in vitro* usually show no altered morphology although they may grow in soft agar.

Work on fibroblasts is considerably more advanced. Fibroblasts, when transformed, give rise to sarcomas, responsible for a minority of human malignant disease. They show, nevertheless, great promise as the basis of a potential screening system for chemical carcinogens. As with bacterial mutation systems, it has often been found necessary to supplement the fibroblast's relatively poor ability to metabolise carcinogens into their active form. This has been achieved, either by cocultivation with other cells capable of carrying out metabolic activation[58], or by isolating the cells from hamster embryos after treatment of the pregnant mother[53].

Much of the work on transformation has been carried out with treatment of mass cultures often for long periods of time and has been subject to some criticism. Transformation has, however, been reported with short treatments followed by cloning[54] and this would seem to be a better approach to adopt in future. A noteworthy feature of much work with both fibroblast and epithelial systems has been the high spontaneous rates of transformation, sometimes considerably higher than one would expect for a gene mutation. Many workers regard this as the result of the artificial environment in which the cells are cultured, and have looked (often successfully) for conditions in which the spontaneous rate is lower. Nevertheless it is likely that the rate of transformation *in vivo* is higher than has been thought and that the body is normally able to deal effectively with the aberrant cells. If this were true the role of DNA-damaging carcinogens might be seen as increasing the already high spontaneous rate and thus overloading the ability of the natural defences of the body to cope with malignant cells.

Most of the work on transformation *in vitro* has concentrated on the development of systems that can be used as models for the study of carcinogenesis (for reviews see refs 55 and 56). To the uninvolved observer, a certain amount of contradiction and inconsistency is apparent. There is, notwithstanding, an impression that this technique may soon be a valued constituent of the battery of techniques for detecting carcinogenic and DNA-damaging substances. Two recent studies have shown successful prediction of carcinogenicity almost as good as that of the bacterial mutagenicity tests[57,14].

Validation

Some of the test procedures described above are very sensitive, but how good are they as predictors of carcinogenicity? Recent reports have presented[35] and discussed[58] carcinogenicity and mutagenicity data obtained for more than 300 chemicals. All the results come from the use of *Salmonella* strains developed by Ames. Where negative mutation results had been obtained with now-obsolete strains, the test was repeated with the latest plasmid-containing strains. Testing was done both without metabolic activation and (generally) with microsomes embedded together with the bacteria in soft agar. With some nitrosamines incubation was carried out with microsomes before plating. A summary of the results is given in Table 1. Since publication, one of the non-carcinogens, 5-hydroxy 2-acetylamino-fluorene, has been shown to be mutagenic because of the presence of an impurity and has now been reclassified as non-mutagenic.

It can be seen that of 179 compounds whose carcinogenic effect on animals is well documented, 157 (or 87.7%) were detected as positive in the bacterial test. This level of confirmation was obtained with essentially all types of compound and was also evident for the small group of compounds for which evidence exists for carcinogenicity in man. The proportion of compounds believed to be non-carcinogenic which gave negative results in the mutagenicity tests was also high: 101 out of 117 (or 86.3%). These included 46 common biochemicals all of which were negative. Seventeen compounds were tested for which carcinogenicity data are uncertain; of these 11 were positive in the mutagenicity test.

The apparent "false" positives and negatives have been discussed elsewhere[58]. It is apparent that many of the latter damage DNA or cause mutations in other systems, or have mutagenic metabolites. Furthermore there is a very obvious limitation to the use of liver microsomes for activation: some chemicals may need reductive activation, or may be metabolised by the gut flora, by organs other than the liver, or by cell components other than microsomes. Indeed it is surprising that liver microsomes are as effective as they seem to be; certainly the method is capable of further improvement.

When one considers the "false" positives, that is the supposed non-carcinogens that register as mutagenic with bacteria, certain difficulties become apparent. Any correla-

Table 1 Correlation of animal carcinogenicity and bacterial mutagenicity with and without metabolic activation (from McCann *et al.*[35])

Group of compounds	Carcinogens detected as bacterial mutagens	Non-carcinogens not mutagenic to bacteria	Compounds of uncertain carcinogenicity detected as mutagens
A Aromatic amines etc.	23/25	10/12	5/7
B Alkyl halides, etc.	17/20	1/3	1/1
C Polycyclic aromatics	26/27	7/9	1/1
D Esters, epoxides, carbamates, etc.	13/18	5/9	0/1
E Nitro aromatics and heterocycles	28/28	1/4	0/2
F Miscellaneous organics	1/6	13/13	0/1
G Nitrosamines	20/21	2/2	1/1
H Fungal toxins and antibiotics	8/9	5/5	–
I Mixtures (cigarette smoke condensate)	1/1	–	–
J Miscellaneous heterocycles	1/4	7/7	–
K Miscellaneous nitrogen compounds	7/9	2/4	–
L Azo dyes and diazo compounds	11/11	2/3	3/3
M Common laboratory biochemicals	–	46/46	–
Total	157/178	101/117	11/17

tion is only as good as the confidence one has in the reliability of both parameters. Whereas positive and negative mutagenicity results can be both unambiguous and reproducible, the same is not true of carcinogenicity results where, as will be discussed below, there are several factors which could result in a failure to detect relatively weak carcinogens. As discussed by McCann and Ames[58], there is good reason to believe that many of the "false" positive chemicals will eventually be shown to be carcinogenic. This has already happened with the food additive furyl furamide which had been used for many years in Japan and had given negative results in two carcinogenicity trials[59]. After positive results had been obtained in *Bacillus subtilis* and *E coli* systems for detecting DNA damage, it was re-examined and shown to produce a low but significant yield for tumours when given to foetal and young mice[60]. There is also the real possibility that some of the "false" negatives are genuine, that metabolism *in vivo* is different from that with isolated microsome preparations. Only further studies in depth can resolve this.

It is worth analysing the data of McCann *et al.*, further to see whether there is any particular type of mutational event (as detected by the *Salmonella*) that is correlated with carcinogenicity. It has been postulated[61] that carcinogenicity is associated with the ability to produce specific types of frameshift mutation This hypothesis does not hold up in any general application. As can be seen from Table 2, whereas most members of some groups of carcinogens (for example, amoratic amines, polycyclic aromatics and nitroaromatics) gave rise to both frameshifts and base-pair substitutions, others (for example, esters, epoxides and carbamates, nitrosamines, miscellaneous nitrogen compounds) gave rise exclusively to base-pair substitutions. There was no group that gave rise exclusively to frameshifts. Taken together, 45.2% of mutagenic carcinogens gave rise solely to base-pair substitutions, 14.8% solely to frameshifts, and 40% gave rise to both.

Rosenkranz (cited in ref. 62) using a Pol⁻ strain of *E. coli* together with the *Salmonella* .set without plasmids, has obtained results as encouraging as those of McCann *et al.* with the plasmid-containing salmonellas. Of about 100 compounds tested, 85% of the known carcinogens were detected (91% of direct acting carcinogens, 72% of procarcinogens). The proportion of non-carcinogens detected as positive was rather high, 30%, but the figure is not comparable with the lower value derived from the data of McCann *et al.*[35] since it did not include the 46 common laboratory biochemicals tested by the latter workers, none of which was positive.

A comparison of the efficiency of various microbial systems for detecting DNA damaging agents has been carried out by Shirasu *et al.*[63]. They found that the hypersensitivity of repair-deficient bacteria (Rec⁻ *B. subtilis*) was the most sensitive. Of 166 pesticides studied, 23 were positive in the Rec-assay (carried out without microsomal activation) Of the 143 negatives, none proved to be positive when tested with *E. coli* or *Salmonella* reverse mutation systems. Of the 23 positives 9 were positive in reverse mutation systems, and of these 9, 1 was not detected by the *E. coli* strains and 1 by the *Salmonella* strains. As far as base-pair substitution mutations are concerned, the non-plasmid *E. coli* strains were found to be preferable to the non-plasmid *Salmonella* strains at least with some groups such as nitrofurans. With other groups such as the organic phosphates a similar small proportion of mutagens was missed by both *S. typhimurium* and *E. coli* strains[64].

The only study in which a single laboratory has compared a number of different tests for predicting carcinogenicity appears to have been carried out by the Central Toxicology Laboratory of ICI (D. Anderson *et al.*, unpublished). The preliminary results with 120 chemicals point to the value of the bacterial mutation tests when metabolic activation is incorporated. The carcinogenicity, or non-carcinogenicity was accurately predicted for 90% of the chemicals by this test. Cell transformation *in vitro* came close with 83% accuracy. Rather less accurate was degranulation of endoplasmic reticulum, 72%, and morphological changes following subcutaneous implantation, 70% correctly predicted Sebaceous gland suppression[65] was good for polycyclic hydrocarbons (90%) but little better than random for other substances (52–62%). Tetrazolium reduction in mouse skin was also poor (62% overall). The authors conclude that some of these rapid tests are capable of distinguishing between carcinogens and non-carcinogens with sufficient accuracy to enable them to be used for selecting potential carcinogens. They also make the point that figures for successful prediction must be treated with

Table 2 Number of carcinogens detected as bacterial mutagens (with or without metabolic activation) classified as to type of mutation induced.

	Base-pair substitutions only	Frameshifts only	Both base-pair substitution and frameshift mutations
A	1	9	13
B	14	2	1
C*	7	5	14
D	13	0	0
E	5	0	23
F	1	0	0
G*	19	0	0
H	0	3	5
I	0	1	0
J	0	1	0
K	7	0	0
L	3	2	6
M	0	0	0
Total	70	23	62

* Data not available for one member.
Key for chemical groups as for Table 1 (From McCann *et al.*[35])

some caution since they can be manipulated within wide limits by the choice of substances tested. As their substances include a large number of non-carcinogenic chemicals closely related to known carcinogens they feel that their results give a reasonably good indication of the likely value of the tests in practice.

DNA damage and human cancer

The correlation between mutagenicity and carcinogenicity is satisfying to those who believe in the somatic mutation theory of cancer[66] and distressing to those who do not[67]. I think the correlation can be more correctly described as being between DNA damaging ability and carcinogenicity. Gene mutation is but one consequence of DNA damage; others such as chromosomal structural rearrangements, virus integration and excision, and changes in gene expression, may well be important in the carcinogenic process. Non-genetic effects are also probably involved.

One could argue that detecting DNA damage is merely a very sensitive way of detecting electrophilic reagents, and that the actual target(s) may well be in other molecules as well as or instead of DNA. This is quite possible; but there is other evidence strongly implicating DNA damage as the rate-limiting step in many carcinogenic processes.

In man, for example, mutations in five complementation groups are known to reduce or abolish the ability of cells to remove ultraviolet photoproducts from their DNA[68]. In all cases they enormously increase sensitivity to the carcinogenic effect of sunlight (resulting in the hereditary disease xeroderma pigmentosum). A further mutation causing the same symptoms has been shown to be associated with a deficiency in another DNA repair pathway active on newly synthesised DNA[69]. Another human mutation responsible for the disease ataxia telangiectasia has been shown to block repair of ionising radiation damage[70] and also results in proneness to develop malignant disease[71]. Thus, the human data strengthen our confidence in the reality of the observed correlation between DNA damaging ability and carcinogenicity. It must be emphasised, however, that even an empirical "litmus paper test", with no known theoretical basis, which gave an 80 to 90% predictiveness for carcinogenicity would be a powerful tool in the screening of chemicals for human toxicity.

The place of tests with mammals

No single test is adequate for a first-tier (sub-mammalian) screen; most authorities agree that a battery of tests must be used as false negatives may occur with any one test. The results of these tests would be used to assign priorities for further testing using mammalian systems. At one extreme, a substance with no apparent effect on sub-mammalian systems might be given a priority so low that no further tests would be considered unless a large human population exposure were to occur or be contemplated. At the other extreme a strongly active substance might well be regarded as hazardous without further testing if the population exposed were small. If it were, say, an industrial chemical, then production workers and users ought to treat it as if it were a known toxic agent or carcinogen, at least until such time as it became possible to carry out full scale animal tests.

The greatest problem in testing for carcinogenicity or mutagenicity with mammals is the insensitivity of most of the tests. This has led, for example, to difficulties in validating microbial carcinogenicity screening systems since many of the "false" positives obtained with these are based on animal experiments that may be inadequate[58]. There have been, and still are, too many carcinogenicity tests with 20 or 30 animals per group.

Provided that the number of animals in each group is kept small, even a large increase in the frequency of neoplasms can fail to be statistically significant and enable a conclusion of "non-carcinogenic" to be drawn (see for example a recent study on the carcinogenicity of hair dyes[72]). As long ago as 1954, Barnes and Denz[73] pointed out that to detect with a probability of 0.01 an effect occurring in 1% of the animals, one would need a group of at least 455 animals. If the effect also occurred spontaneously then the number of animals per group would have to be increased manyfold. Today, notwithstanding, carcinogenicity experiments with 100 animals per group are often regarded as "good" and those with 200 animals per group are extremely rare. But although "kilomouse" experiments are theoretically attractive there may be little to be gained from them in practice. Logistical problems dictate that such experiments be phased over many weeks and involve slightly varying conditions. "Spontaneous" rates of tumour occurrence unfortunately often vary in time and place, perhaps reflecting slight differences in diet, and it is often difficult to run an adequate control group. Errors in handling are also more likely in very large experiments.

Whereas a significant reproducible positive result in a mammalian test may be taken as indicating the existence of a potential hazard for man, a negative result taken should not necessarily be taken to indicate the absence of hazard, particularly if the human population to be exposed is very large, and the number of animals in the test small. We may take some comfort where the disparity in dose between the animal and human exposure is great. This is not always so. Anaesthetic gases, for example, are given to an appreciable fraction of the population in Western society at concentrations which are not far from the lethal level. One might well feel that a negative result in a screening test with a few dozen mice would be of little value.

It can be seen that mammalian tests are not wholly appropriate for the validation of sub-mammalian tests, and it is perhaps remarkable that they should show such good agreement Validation of one type of test against another must not blind one to the real objective, which is to predict long term toxic effects in man. There are few proven human carcinogens and most of these can be detected by both mammalian and sub-mammalian tests. In man, carcinogens tend to be recognised only when the tumour is of a rare type and there is a sufficient cluster of cases to enable association with a particular occupation to be seen by an alert clinician. Genetic effects in man are even harder to detect retrospectively. There are several examples of somatic chromosome damage in lymphocytes of persons exposed to known mutagenic and carcinogenic substances (for example, vinyl chloride[74], ozone[75], benzene[76], toluene[77], cadmium[78,79] and methyl mercury[80]. Recent evidence for the possibility of dominant lethal damage in man by vinyl chloride (P. Infante, unpublished) and anaesthetic gases[81] is ominous but needs closer examination.

Ultimately quantitative risk assessments must be attempted, for we must face the unpalatable fact that man will almost certainly have to be exposed to some carcinogens and mutagens whose benefits cannot be dispensed with and others which it is impracticable to eliminate from the environment. Risk evaluations at the present time almost always require information which is not available, such as the nature of the dose–effect response at low doses. Nevertheless, approaches must be found that will lead eventually to risk–benefit evaluations based less on guesswork and more upon knowledge. In the meantime the use of short term tests would enable potentially carcinogenic substances to be identified among the many thousands for which long term animal testing cannot at present be contemplated. This in turn would open the door to provisional regulatory action to minimise human exposure. Taken seriously and on a large enough scale, there is good reason to believe that this approach would ultimately result in a reduction in the incidence of chemically induced cancer.

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articles

Isolation and N-terminal amino acid sequence of membrane-bound human HLA-A and HLA-B antigens

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Membrane-bound HLA-A and HLA-B antigens have been extensively purified in good yield. The sequences of the N-terminal 16 amino acids have been determined using about 1 nmol of protein eluted from polyacrylamide gel after electrophoresis in sodium dodecyl sulphate.

THE major histocompatibility region of man (HLA) occupies at least 1 to 2 recombination units on chromosome 6 and probably contains a large number of genes involved in diverse immune and possibly other functions[1]. The gene products that have so far been identified with this region comprise various

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Table 1 Purification of HLA-A2 antigen from BRI 8 cells

Fraction	Protein (% recovery)	HLA-A2 activity (% recovery)	Degree of purification of HLA-A2
BRI 8 cells	100	100	1
Plasma membrane	1.0	46	45
Na deoxycholate-soluble	0.95	38.8	41
Gel-filtration	0.26	36.1	136
Glycoprotein	0.026	32.5	1,240

BRI 8 cells grown by Searle Diagnostic were used as the antigen source. The cells (about 80% viable; 5×10^7 cells ml^{-1} of 10 mM Tris-HCl buffer-0.15 M NaCl, *p*H 7.4) were broken using the Stansted cell disruptor (model A0 612; disrupting valve 516; air pressure applied to disrupting valve 12 p.s.i.). The plasma membrane fraction was separated, as previously described[15], by differential centrifugation and discontinuous sucrose density gradient centrifugation (36% (w/v) against 25 (w/v) sucrose as judged by refractive index measurement), and was washed by homogenising twice in 10 mM Tris–HCl buffer, *p*H 7.3. The membrane (4 mg of protein ml^{-1}) was solubilised at 0 °C by addition of sodium deoxycholate (10% (w/v) in 10 mM Tris–HCl buffer, *p*H 8.2) to a final concentration of 2% and was immediately centrifuged at 100,000*g* for 1 h. The supernatant (7 ml) was subject to gel filtration on a column (55 × 5.0 cm) of Ultragel AcA 34 (LKB Instruments) in 0.5% deoxycholate at a flow rate of 70 ml h^{-1}. The fractions corresponding to the A and B antigens (see Fig. 2, ref. 16) were pooled (about 60 ml) and added to a column (16 × 1.6 cm) of *L. culinaris* lectin–Sepharose 4B (1 mg of lectin per ml of gel sediment) in 0.5% Na deoxycholate. The bound glycoproteins were subsequently eluted with 2% methyl-α-D-mannopyranoside in 0.5% Na deoxycholate (see Fig. 2, ref. 15). The results shown were obtained using lectin isolated from lentils purchased from Whitworths. Similar results have been consistently obtained using lentils from this source, but a few batches of lentils from other sources have given lectin that bound a portion of the HLA-A and -B activities only. A, B and C antigen activities were measured by inhibition of cytotoxicity[18] using antisera which have been previously described[16]. The purification of the A1, B8 and B13 antigens was similar to that illustrated for the A2 antigen.

cell surface antigenic determinants as well as the C2 (ref. 2), C′3 proactivator[3], C4 (ref. 4) and C8 (ref. 5) components of complement. The most extensively characterised cell surface antigens are those of the three highly polymorphic segregant series of the HLA-A, -B and -C loci [1,6]. The HLA region also codes for a set of serological specificities, called Ia (immune-associated) antigens[7]. These probably represent the products of several loci (refs 7–10 and J. G. Bodmer, personal communication), some of which show a close association with the HLA-D locus that controls the expression of the major determinants involved in mixed lymphocyte responses[11]. The functions of these loci have yet to be delineated but they seem to be connected with the ability to generate immune responses against foreign substances[12], tumours and virus-infected cells[13] as well as other phenomena related to cell–cell recognition. A complete understanding of the biological relevance of these gene products relies ultimately upon their isolation in a highly purified state and the determination of their structure.

The choice of tissue for the isolation and subsequent structural studies of the HLA-A, -B, -C and Ia antigens has been dictated by several considerations. HLA-A, -B and probably -C locus antigens are expressed on the surface of almost all cell types except erythrocytes. Ia antigens, however, show a much more restricted tissue distribution, being found on B lymphocytes, monocytes and possibly some endothelial and epithelial cells, but not T lymphocytes and fibroblasts[14]. Our choice of cultured human lymphoblastoid B cell lines as the tissue source has not only enabled the simultaneous purification of the HLA-A, -B and Ia antigens, but has also permitted the purification of antigen of restricted and reproducible specificity in amounts sufficient for structural studies.

The selection of purification procedure was influenced by various factors such as HLA-A and -B; Ia antigens are integral plasma membrane glycoproteins and being insoluble in conventional aqueous solvents need to be solubilised in detergents[15]. In particular, the fractionation techniques were selected because they gave a high yield coupled with considerable discrimination. This was necessary because of the high cost of cultured cells and because the HLA-A and -B antigens comprise <0.1% of the total cell protein. The separation of the cell surface (plasma) membrane was chosen as the initial purification step in preference to either the preparation of a crude membrane fraction or the extraction of whole cells with detergent. The reasons for choosing this approach were that it greatly reduced the possibility of degradation and of the generation of structural artefacts, such as dimerisation through disulphide interchange[16], as well as giving a significant degree of purification. The success of this approach depends, however, on being able to prepare highly purified plasma membrane in good yield (preferably better than 50%).

The structural analysis was facilitated by the availability of a new microtechnique for determining N-terminal amino acid sequences (J. B. unpublished). This technique combines high sensitivity with the considerable resolving power of SDS–polyacrylamide gel electrophoresis, and is applicable to less than 1 nmol of protein eluted from polyacrylamide gels.

Purification of HLA-A and -B antigens

The purification of HLA-A2 antigen from the lymphoblastoid B cell line BRI 8 (HLA specificities: A1, A2, B8, B13 and CW2) is summarised in Table 1. The A1, B8 and B13 antigens co-fractionated with the A2 antigen and were purified to

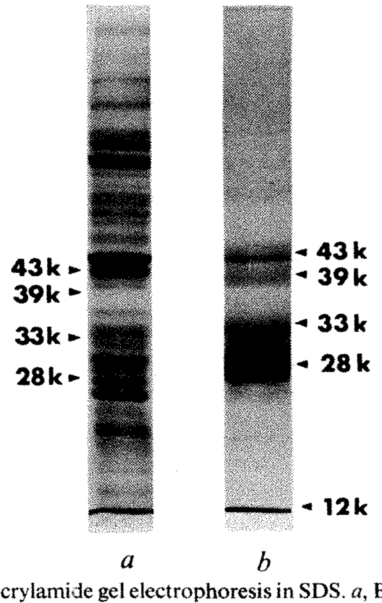

Fig. 1 Polyacrylamide gel electrophoresis in SDS. *a*, BRI 8 plasma membrane; *b*, glycoprotein fraction (Table 1). Electrophoresis was performed in 10% (w/v) polyacrylamide slab gels using the Tris–glycine discontinuous buffer system of Laemmli[21]. Samples were dissolved by heating at 100 °C for 2 min in 2% (w/v) SDS, 0.1 M dithiothreitol, 10% (w/v) glycerol and 0.02% bromophenol blue. Gels were stained with 0.1% Coomassie blue in methanol, water, acetic acid (41:52:7, by volume) and destained with the same solvent. Molecular weights were calculated by reference to the mobilities of standard proteins: transferrin (78,000), bovine serum albumin (69,000), ovalbumin (45,000), immunoglobulin L chain (25,000), soybean trypsin inhibitor (21,000) and cytochrome *c* (12,500). In the above conditions β_2-microglobulin was coincident with the bromophenol blue band at the gel front. The presence in the glycoprotein fraction of a band with a molecular weight of 12,000 was revealed by using 12% (w/v) polyacrylamide gels.

similar extents. The yield and purity of the purified plasma membrane is intimately related to the method used for cellular disruption[17]. In this study the cells were broken using a commercial model of the cell-disrupting pump (Stansted Fluid Power). The plasma membrane was separated by differential centrifugation and discontinuous sucrose density gradient centrifugation[16] and was washed by homogenising under hypotonic conditions. Under these conditions yields of purified plasma membrane in excess of 50% are often achieved. The purified membrane was solubilised in sodium deoxycholate before purifying the A and B antigens by conventional separation techniques such as gel filtration and affinity chromatography. Sodium deoxycholate (final concentration 2%) proved superior as the solubilising agent to other weakly anionic detergents such as Ammonyx Lo, and the non-ionic detergents Nonidet P-40 and Triton X-100. It was selected in preference to the strongly ionic detergents such as SDS because, in contrast to these detergents, it had a negligible effect on antigenicity and did not interfere with specific molecular interactions[19]. The solubilised membrane was fractionated by gel filtration on Ultragel AcA 34 in sodium deoxycholate. Ultragel AcA 34 was used in preference to Sephadex G-200 and Bio-Gel P-150 because of its superior column stability and flow rate. The glycoproteins of the gel filtration fraction were next separated from the non-glycosylated proteins by selective adsorption to *Lens culinaris* lectin–Sepharose 4B in sodium deoxycholate and subsequent elution with methyl-α-D-mannopyranoside. Gel filtration was selected to precede affinity chromatography since adsorption to and elution from *L. culinaris* lectin–Sepharose provided a very convenient concentration step. As shown in Table 1 the overall purification procedure gave a 1,240-fold increase in the specific activity of the A2 antigen relative to BRI 8 cells with a recovery of 33%.

The CW2 antigenic activity of BRI 8 cells co-fractionated with the A and B antigens up to the gel filtration step but was not detected in the glycoprotein fraction. The results suggest that it was not adsorbed to *L. culinaris* lectin–Sepharose due either to a different carbohydrate composition (compare the behaviour of rat thymocyte Thy-1 antigen in ref. 20) or to the absence of carbohydrate.

The glycoprotein fraction was recovered by precipitation at −20 °C for 48 h with 66% (v/v) ethanol in which deoxycholate and sugar are soluble, and was analysed by polyacrylamide gel electrophoresis in SDS. Figure 1 shows that the glycoprotein fraction has a very restricted polypeptide composition compared with that of the original plasma membrane preparation. Only five bands whose position corresponded to molecular weights of 43,000, 39,000, 33,000, 28,000 and 12,000 were detected. Various results indicate that the 43,000 and 12,000 molecular weight chains are derived from the HLA-A and -B antigens. This conclusion is based on the reports[22–24] that the A and B antigens are composed of two non-covalently-bound polypeptide chains with molecular weights of about 43,000 and 12,000, and that the smaller chain is identical with β_2-microglobulin. It is also supported by the observation that only these two chains were specifically adsorbed when the glycoprotein fraction (Table 1) was added to a column of antibody to β_2-microglobulin attached to Sepharose and were subsequently eluted by 3 M potassium thiocyanate in 0.5% sodium deoxycholate, *p*H 8.5 (ref. 25).

The remaining polypeptide chains are of unknown function, although the following evidence suggests that those of molecular weight 33,000 and 28,000 represent the human counterparts of the mouse Ia antigens. First, the polypeptide chains of mouse Ia antigens possess similar molecular weights of about 30,000 (ref. 26). Second, the Ia allogeneic activity expressed on the surface of BRI 8 cells co-fractionated with the A and B antigens on gel filtration and *L. culinaris* lectin–Sepharose[27]. Third,

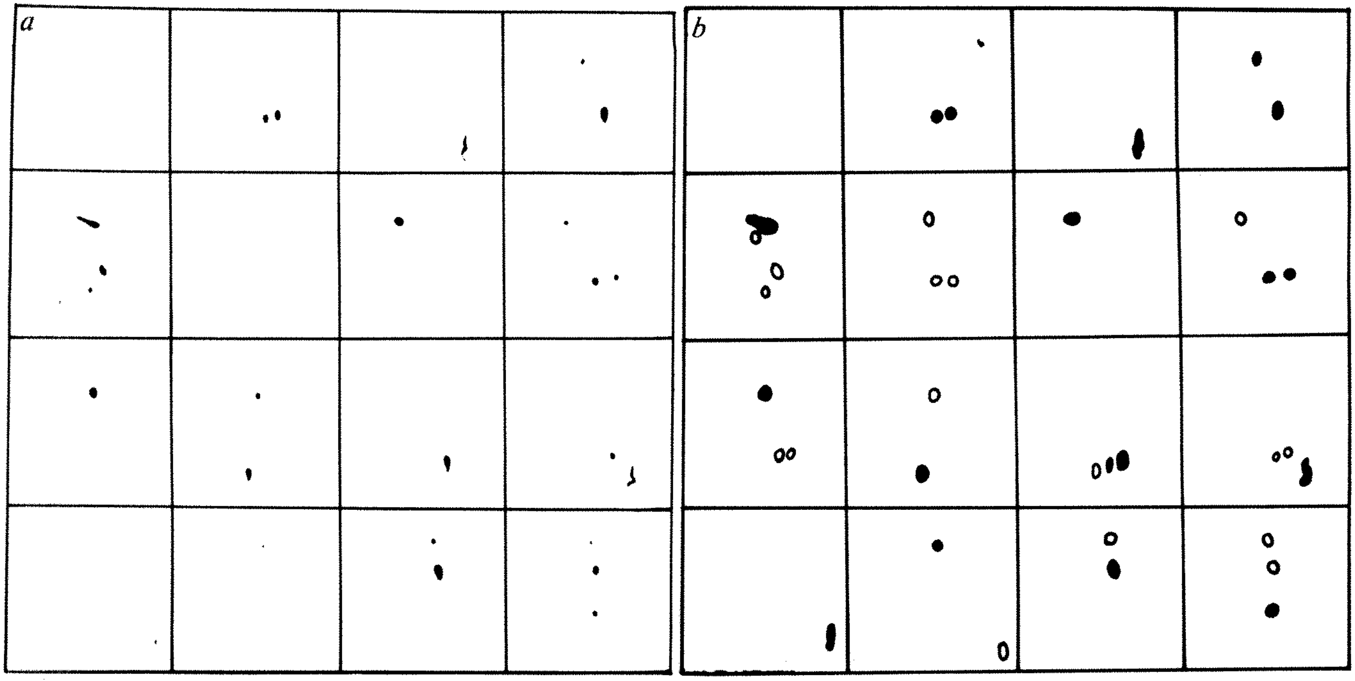

Fig. 2 *a*, Autoradiograph of thin-layer chromatograms resulting from sequence analysis of the 12,000 molecular weight polypeptide chain recovered after polyacrylamide gel electrophoresis of the glycoprotein fraction in sodium dodecylsulphate (SDS). Phenylthiohydantoin–amino acids were separated on 5×5 cm polyamide layers by 2-dimensional chromatography[33] using 5-*bis* [2(5-*tert*-butylbenzoxazolyl)]-thiophene (0.1%) as fluorescent indicator[34]. The phenylthiohydantoins of histidine and arginine were resolved by redeveloping the chromatograms in the direction of the first solvent using *n*-propanol, formic acid (20:1, v/v). Radioactive spots were revealed by autoradiography using Kodak 'Auto-process' X-ray film and were identified relative to the unlabelled internal markers that were visualised under ultraviolet light. The autoradiograph was exposed for 60 h. The variation in intensity of the spots at successive steps partly reflects the varied amount of cyclised derivatives analysed. The residues are arranged in sequence from left to right. The sequence shown begins at residue no. 2 since residue no. 1 remains attached to the resin and is not identified. The blank at residue no. 6 indicates that no information is available on this residue although, by implication, it is lysine which would remain attached to the resin and consequently would not be identified. The origin of the background of faint spots that increased in intensity with increase in the number of steps is not clear. This background was independent of the protein analysed (see Fig. 3). In the above conditions phenylthiohydantoin–glutamine (residues nos 2 and 8) and phenylthiohydantoin–serine (residue no. 11) give two spots due to decomposition (see also Fig. 3, residues nos 2 and 4). *b*, Drawing of autoradiograph. The filled-in areas correspond to the spot(s) due to the amino acid(s) identified at each step. The open circles represent spots which were detected but which were judged to be due either to overlap from the previous residue(s) or to background.

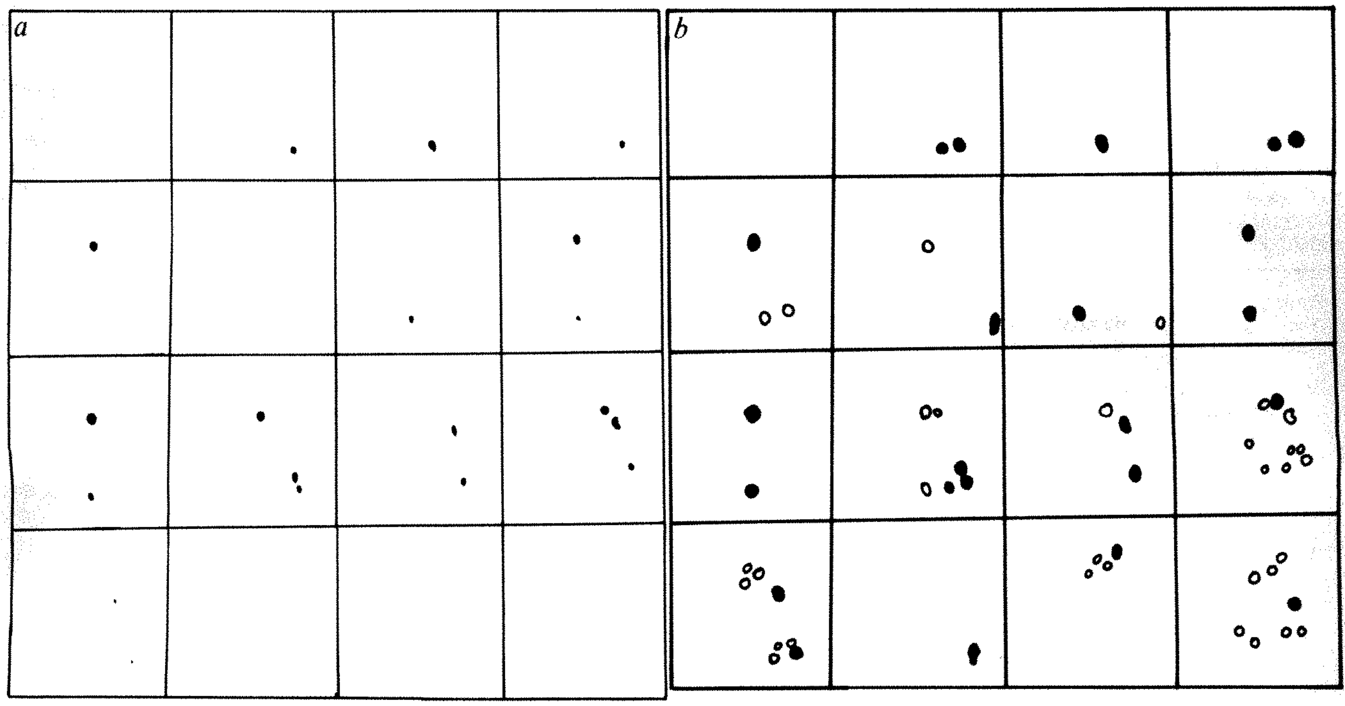

Fig. 3 *a*, Autoradiograph of thin-layer chromatograms resulting from sequence analysis of the 43,000 molecular weight polypeptide chain recovered after polyacrylamide gel electrophoresis of the glycoprotein fraction in SDS. Experimental details were as described in the legend to Fig. 2 except that the autoradiograph of the first twelve residues was exposed for 60 h. and that of residues nos 13–16 for 120 h. The sequence determined from the data is shown in Table 2. *b*, See Fig. 2*b* legend.

antisera produced in rabbits and mice against the 33,000 and 28,000 polypeptide chains of the glycoprotein fraction had many of the properties expected for heterologous anti-Ia sera[27,28]. Thus, they reacted preferentially with human B-lymphoblastoid cell lines, purified peripheral blood B lymphocytes and monocytes, but not T cell lines, peripheral T cells, fibroblasts and erythrocytes. The immunoglobulin fraction of the antisera and its Fab fragments also blocked human mixed lymphocyte cultures[27].

Amino acid sequence of A and B antigens

The N-terminal amino acid sequences of the 43,000 and 12,000 molecular weight chains were determined by the high-sensitivity solid-phase procedure[29] using material extracted from the polyacrylamide gel (J. B., unpublished). As soluble foreign material occluded in the gel, and the glycine of the Laemmli buffer system[21] interfered with the subsequent procedure, the following separation method was adopted. The polyacrylamide gel (15%, w/v) slab (8×7×0.15 cm) was washed overnight in 0.17 M Tris-HCl buffer, *p*H 8.15, containing 0.1% SDS, reinserted into the gel apparatus and cut to size before casting a 3% (w/v) polyacrylamide stacking gel on to the top edge. The glycoprotein fraction (400 µl containing 100 µg of A and B antigens in 2% SDS, 0.1 M dithiothreitol, 10% glycerol and 0.02% bromophenol blue) was added and electrophoresis was carried out using the Trisborate discontinuous buffer system of Neville[30]. The edges of the gel were detached, stained for 30 min with 0.1% Coomassie blue and destained for 90 min in methanol–water–acetic acid (41:52:7, by volume) followed by 10 min in 7% (v/v) acetic acid. Using the stained strips as guides the regions corresponding to the 43,000 and 12,000 molecular weight chains were removed, cut into 2-mm cubes and extracted overnight by shaking at 37 °C in 1 ml of 0.04 M *N,N'*-dimethyl-*N*-allylamine buffer in pyridine, water (3:2, v/v) (Pierce Chemical Co.) containing 0.1% SDS and buffered to *p*H 9.5 with trifluoroacetic acid. The eluted protein was covalently attached to *N*-(2-aminoethyl)-3-aminopropyl glass (120 Å pore diameter) that had been activated by treatment with phenylene diisothiocyanate in dimethylformamide[29].

Amino acid sequences were determined on an Anachem SPA 1200 automatic solid-phase sequencer using high specific activity ^{35}S-labelled phenylisothiocyanate (2% (v/v) solution in acetonitrile containing 1 mCi ml^{-1}; Radiochemical Centre, Amersham) for the coupling. The reaction was completed by a second coupling with unlabelled phenylisothiocyanate (5% solution in acetonitrile). The cleaved thiazolinones were collected into tubes containing all the conventional phenylthiohydantoin-amino acids (1 nmol of each in 10 µg of ethyl acetate) to act as carrier and markers. The thiazolinones were converted to thiohydantoins by heating at 80 °C for 10 min in 200 µl of 20% aqueous heptafluorobutyric acid. The phenylthiohydantoin-amino acids were extracted with ethyl acetate and were identified by two-dimensional thin-layer chromatography.

The applicability of the method is evident from Fig. 2 which shows the autoradiograph obtained for the N-terminal 16 residues of the 12,000 molecular weight polypeptide chain of the glycoprotein fraction. The amino acid sequence is clearly discernible as:

1 5 10
— -Gln–Arg–Thr–Pro– — -Ile–Gln–Val–Tyr–
15
Ser–Arg–His–Pro–Ala–Glu–

This sequence is, apart from the blanks at residues no. 1 and 6, identical with that determined previously by Cunningham and coworkers[33] using conventional techniques, for the N-terminus of human urinary β_2-microglobulin.

The autoradiograph of the N-terminal 16 residues of the 43,000 molecular weight polypeptide chain is shown in Fig. 3. The identification of the spots was straightforward apart from that of residue 3 which could not be related to any known phenylthiohydantoin-amino acid; this residue was also not identified by conventional sequencing methods (Table 2). One possible explanation is that this residue is substituted by carbohydrate. The interpretation of the sequence was less straightforward than that of β_2-microglobulin. This was due to the heterogeneity of the 43,000 molecular weight polypeptide

Table 2 Comparison of N-terminal sequences

Protein	Antigenic specificity	Residue no. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
43,000 molecular weight chain	A1, A2								Phe	Phe	Thr	Ala		Ala			
			Ser		Ser	Met	Arg	Tyr					Val		Arg	Pro	Gly
	B8, B13								Tyr	Tyr	Ser	Ser		Ser			
Papain-solubilised	A2	Gly	Ser		Ser	Met	Arg	Tyr	Phe	Phe	Thr	Ser	Val	Ser			Gly
34,000 molecular							Arg										
weight chain	B7, B12	Gly	Ser		Ser	Met	Val	Tyr	Phe	Tyr	Thr	Ala	Val	Ser	Arg	Pro	Gly

Amino acid sequence of the N-terminal 16 residues of the 43,000 molecular weight polypeptide chain compared with the N-terminal sequences of the larger polypeptide chains of the papain-solubilised HLA-A2, and mixed HLA-B7 and HLA-B12 antigens reported by Terhorst and coworkers[34]. The A and B antigenic specificities of the 43,000 molecular weight polypeptide chain are based on the known specificities of the glycoprotein fraction and on reports that the allogeneic A and B specificities are associated exclusively with a polypeptide of this molecular weight[24]. The sequence of the 43,000 molecular weight chain is based on the results shown in Fig. 3 and begins at residue 2 since residue 1 remains attached to the resin and is not identified. Blank positions indicate that no information is available on the residue at this point. Residue 3 of the 43,000 molecular weight chain gave a detectable spot on thin-layer chromatography (Fig. 3) but this spot could not be identified with any known phenylthiohydantoin-amino acid.

chain (A and B antigens with polymorphic specificities) and to the need to differentiate between the presence of more than one residue at a particular position and overlap from the previous residue, especially when the same amino acid occurred at adjacent positions (residues 8 and 9, for example). Overlap was judged on the basis of experience gained from the analysis of unique sequences (J. B., unpublished). The most probable sequences are presented in Table 2 together with the N-terminal sequence of the papain-solubilised HLA-A2, and mixed HLA-B7 and HLA-B12 antigens determined using conventional methods[34].

It is apparent that the solid-phase sequencing method has much to recommend it. It is applicable to < 1 nmol of protein eluted from polyacrylamide gel after electrophoresis in SDS and is rapid (1 cycle (residue) h^{-1}). As with conventional methods the extent of sequence attainable is limited by decrease in yield, increase in background and overlap from the previous residue. The sequence of β_2-microglobulin could have been extended beyond residue 16, but little further information was obtainable from the 43,000 molecular weight chain.

Polymorphism

Clearly the N-terminal sequence of the 43,000 molecular weight polypeptide chain represents the sum of the sequences of the papain-solubilised A and B antigens, apart from some slight variation at residues 6, 8, 10 and 13. The marked similarity between the N-terminal sequences indicates that papain must cleave the polypeptide chain towards the C-terminus and that the N-terminus is exposed on the cell surface. This means that the portion of the polypeptide chain inserted into the lipid bilayer of the membrane is close to the C-terminus, although it does not rule out the possibility that the actual C-terminus is exposed on the inner or outer face of the membrane. The same conclusions have been drawn for the homologous mouse H-2 antigens[35]. Similarly, it is apparent from the identity of the N-terminal sequences of the membrane-associated and urinary β_2-microglobulins, that if the urinary material has been degraded then cleavage must have occurred towards the C-terminus.

If, as seems likely, the HLA-C locus products were not present in the material used for sequence analysis, then several interesting points emerge from the data in Table 2. First, at least 10 of the N-terminal 16 residues are apparently constant for both the A and B locus antigens, and all of the amino acid variations correspond to single nucleotide base changes. This restricted heterogeneity is consistent with the suggestion that the A and B genes arose from a common ancestral gene by duplication[1,36]. In this context the sequence of the C antigen will be particularly informative since, although its molecular size and shape in deoxycholate resemble more closely those of the A than the B antigens (ref. 16 and C.B., W.F.B., M.J.C., and D.S., unpublished), the strong linkage disequilibrium between the C and B locus determinants suggest that the C gene(s) arose from the B gene(s)[36]. Second, the sequence differences at residues 9 and 11 can be interpreted as A and B locus differences, but those at residues 6, 10 and 13 cannot be interpreted in this way. Thus, the A1, A2, B8 and B13 antigens collectively have arginine at residue 6 whereas the B7 and B12 antigens have arginine and valine. This variation is consistent with the suggestion that the B7 or B12 antigen differs from other A and B antigens in having valine at this position. Similarly, the serine and alanine residues at positions 10 and 13 respectively may represent changes that are directly related to the A1, B8 and/or B13 specificities. If these and other changes do contribute to the serologically defined polymorphism then each allele may represent the sum of a number of changes at positions remote from each other in the unfolded polypeptide chain. The present data are too limited to provide an answer to this proposal or to the postulated common origins of histocompatibility antigens and immunoglobulins[1,37]. Definitive answers will, however, emerge as more sequence data are accumulated. It will, in general, be interesting to delineate the evolutionary relationships between the products of the different alleles and loci of the HLA system, and, in particular, to see whether cross-reacting antigens (A2 and A28, for example) have some common changes that distinguish them from all other antigens as well as additional changes that distinguish them from each other.

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Cell-cycle, cell-shape mutant with features of the G_0 state

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A cold-sensitive mutant of CHO cells has features of "reverse transformation" at the non-permissive temperature of 33 °C. Cells accumulate at G_1 with altered morphology and remain viable and quiescent for more than 40 d. Such cultures are synchronised by a temperature shift back to the permissive 39 °C.

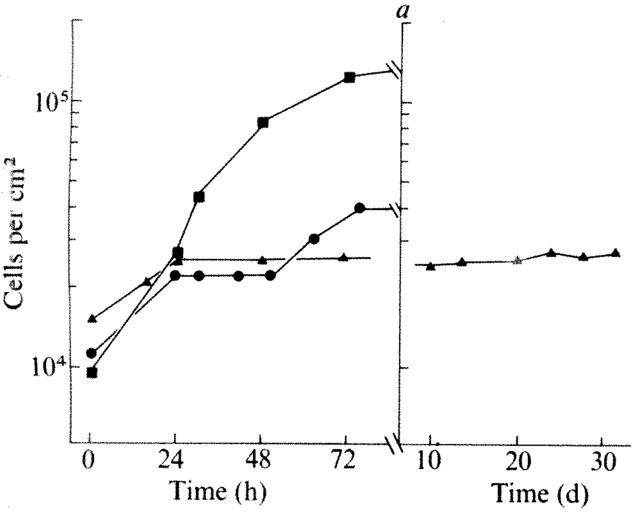
THE selection and isolation of temperature-sensitive mutants of eukaryotic cells defective at a specific phase of the cell division cycle (cdc) provides an attractive model for the study of growth control *in vitro*. Hartwell *et al.*[1,2] have pioneered this approach by isolating 150 *cdc* mutants of the yeast *Saccharomyces cerevisiae* defective in the initiation of DNA synthesis, DNA replication, bud emergence and cytokinesis. More recently, heat-sensitive mutants of mammalian cells have been described including variants defective at metaphase[3], G_1[4–6], DNA replication[7,8], and cytokinesis[9]. Most mammalian *cdc* mutants, however, are disappointing in two important respects: rapid loss of viability at the non-permissive temperature and failure to be synchronised by a temperature shift.

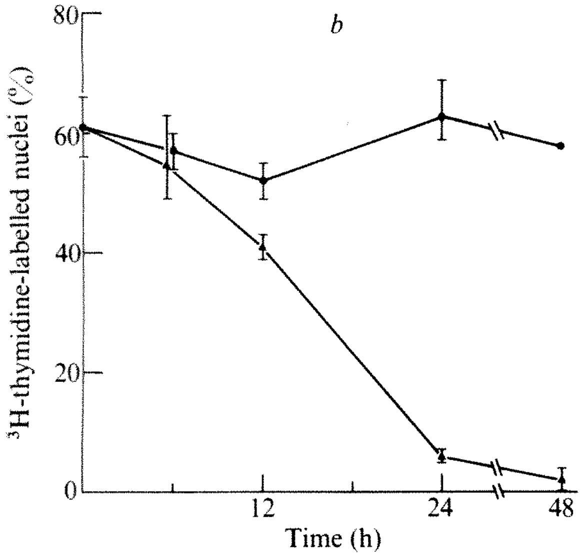
We describe here a cold-sensitive mutant of Chinese hamster ovary cells, clone cs4-D3 initially isolated and provided by Dr Rosann Farber, which has unique features at the non-permissive temperature (33 °C). First, cells accumulate at the G_1 interval and remain viable and quiescent indefinitely. So far, we have maintained the mutant at 33 °C for 40 d without loss of viability or potential for growth at the permissive 39 °C. Second, there is a cell-cycle-dependent change in morphology: cells are transformed from their normal "fibroblast-like" shape to a round, epithelial form. This shape change can be reversed at the non-permissive temperature by addition of dibutyryl (db) cyclic AMP. Finally, cells can be synchronised by a temperature shift (39 °C to 33 °C to 39 °C) and then undergo several rounds of synchronous division. This is the first report of a cell cycle mutant reversibly arrested in a quiescent state. Clone cs4-D3 may provide a useful *in vitro* model for the study of the induction of DNA synthesis and the relationship between cell morphology and cell growth.

Selection and isolation of cold-sensitive mutants were as reported before[10]. Briefly, CHO cells were mutagenised with ethyl methane sulphonate and selected at 33 °C by the BUdR–visible light technique. Stock cultures of cs4-D3 were maintained at 39 °C as monolayers (Falcon culture flasks; 25 cm^2 surface area) in Eagle's basal diploid medium—10% foetal calf serum supplemented with non-essential amino acids and passaged twice weekly at a split ratio of 1:20. Experimental cultures were established in disposable glass scintillation vials (surface area 4.3 cm^2; 2–4×10^4 cells) and maintained at a constant temperature (± 0.1 °C) in a water bath fitted with a contact thermometer (Grant Instruments, Cambridge Ltd).

When exponentially growing cultures of cs4-D3 were stepped down to the non-permissive temperature (39–33 °C) cell numbers increased by about one population doubling to plateau values (Fig. 1*a*; division index, 0.6–0.9) and the number of ^{3}H-thymidine-labelled nuclei fell to base line (<2% at 48 h; Fig. 1*b*), while samples established at a similar density from stationary cultures increased no further in cell number. In contrast, growth of wild-type CHO cells was only marginally reduced at 33 °C (Fig. 1*c*). cs4-D3 remained viable at the non-permissive temperature for a minimum of 40 d, as determined by dye exclusion tests (>95%) and showed no sign of detachment from the glass surface. Moreover, cells retained the ability to grow at the permissive temperature (see below and Fig. 3). In long term experiments (>5 d) this was routinely tested at 2-d intervals by "stepping up" replicate cultures and measuring increases in cell number. The mutant was "leaky" at the slightly higher temperature of 34 °C and, after an initial growth plateau, there was a further increase in cell number by 72 h (Fig. 1*a*). This emphasised a need for strict temperature control (33±0.1 °C) of all experimental cultures.

Change in morphology was perhaps the most striking feature of this mutant. At the permissive temperature, cs4-D3 resembled a fibroblast with an elongate, spindle shape and smooth surface membrane (Fig. 2*a*) whereas at 33 °C most cells (>90% by 24 h) were round and epithelial-like with a rough surface membrane bearing knob-like protrusions (Fig. 2*b*). Moreover, the epithelial form assumed by the mutant at the non-permissive temperature could be reversed by the addition of db cyclic AMP (Fig. 2*c*). This is shown in the following experiment. Coverslip cultures were incubated for 48 h at 33 °C (>90% epithelial-like cells at 48 h) before treatment with 0.5 mM or 1 mM dibutyryl cyclic AMP. Thereafter, replicate samples were fixed at intervals in 0.1% (v/v) glutaraldehyde and examined by light microscopy (Table 1). At 6 h, cells treated with db cyclic AMP (Fig. 2*c*) could not be distinguished from corresponding controls maintained at 39 °C. Cells had reverted to an elongate, spindle shape and showed no further sign of surface "blebbing". In spite of this





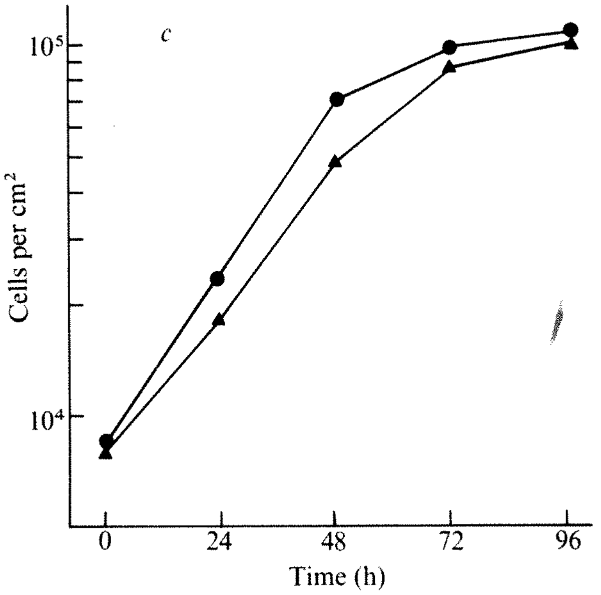


Fig. 1 *a*, Growth of cs4-D3 at different temperatures. Cells (0.5–1 × 10^4; 2 ml) were seeded in screw-top glass vials (surface area, 4.3 cm^2) and incubated for 48 h at 39 °C for exponential growth. Cells were then cultured further at 39 °C (■), 34 °C ± 0.1 °C (●) or 33 °C ± 0.1 °C (▲). At the times indicated, samples were trypsinised and cell numbers were estimated in a Coulter counter, model 2B1; values represent the mean for five determinations. *b*, Percentage of nuclei labelled with ^{3}H-thymidine at 39 °C (●) or 33 °C (▲). Coverslip cultures of cs4-D3, in the exponential phase of growth, were incubated at either the permissive 39 °C or the non-permissive 33 °C. At the times indicated, triplicate samples were exposed to 6-^{3}H-thymidine (0.1 μCi ml^{-1}; 5 Ci mmol^{-1}) for 30 min, washed in EBD medium, and fixed for 10 min in acetic acid–ethanol (1:3, v/v). Air-dried coverslips were mounted on microscope slides and autoradiographs were prepared using Ilford K5 nuclear emulsion. *c*, Growth of wild-type CHO cells, determined as in (*a*) at 33 °C (▲) and 39 °C (●).

dramatic change in shape there was no effect of db cyclic AMP (0.05–1 mM for 24–72 h) on growth and cells remained quiescent at 33 °C.

Recent studies by time-lapse cinematography (our work with J. B. Clarke, in preparation) indicate that change in shape is dependent on the cell cycle: at 33 °C cells maintained their fibroblast appearance throughout the intermitotic period, rounded up for mitosis and divided but then failed to spread out on the substratum. They remained in this rounded shape indefinitely but were still firmly attached to the culture flask. Conversely, when cells were returned to 39 °C, normal morphology was only observed after mitosis. This delay of one cell cycle may be of interest from a development aspect.

A similar change of shape in the reverse direction, from epithelial to fibroblast, has been described by Puck *et al.*[11–13] for a variant of wild-type CHO cells, clone K1, after treatment with db cyclic AMP or certain prostaglandins. An interesting comparison can be made between their published illustrations and those presented here: wild-type CHO-K1 has an identical shape to the mutant at the non-permissive temperature (Fig. 2*b*) while dibutyryl cyclic AMP-treated cultures of CHO-K1 resemble cs4-D3 at 39 °C (Fig. 2*a*). Puck *et al.* have introduced the term "reverse transformation"[13] to describe the sum of effects induced by db cyclic AMP: change in shape, ordered arrangement of confluent cultures, decreased agglutination with plant lectins, increased synthesis of collagen—the changes mirrored those found in neoplastic transformation. Cell morphology, however, was unrelated to growth behaviour and the generation time in either epithelial or fibroblast form was similar. Clone cs4-D3 illustrates a further distinction between cell shape and cell growth by resembling a normal fibroblast at 39 °C and a virally transformed cell at 33 °C.

The effects of db cyclic AMP on cell shape are antagonised by Colcemid and vinblastine, thereby implicating microtubules; this has received some support from ultrastructural studies[14]. We have no evidence for the involvement of microtubules in the cold-labile defect, and preliminary analysis by electron microscopy showed no significant difference in microtubule content of cs4-D3 between 33 and 39 °C.

To determine whether the mutant was defective in a specific phase of the division cycle, "labelled-mitoses

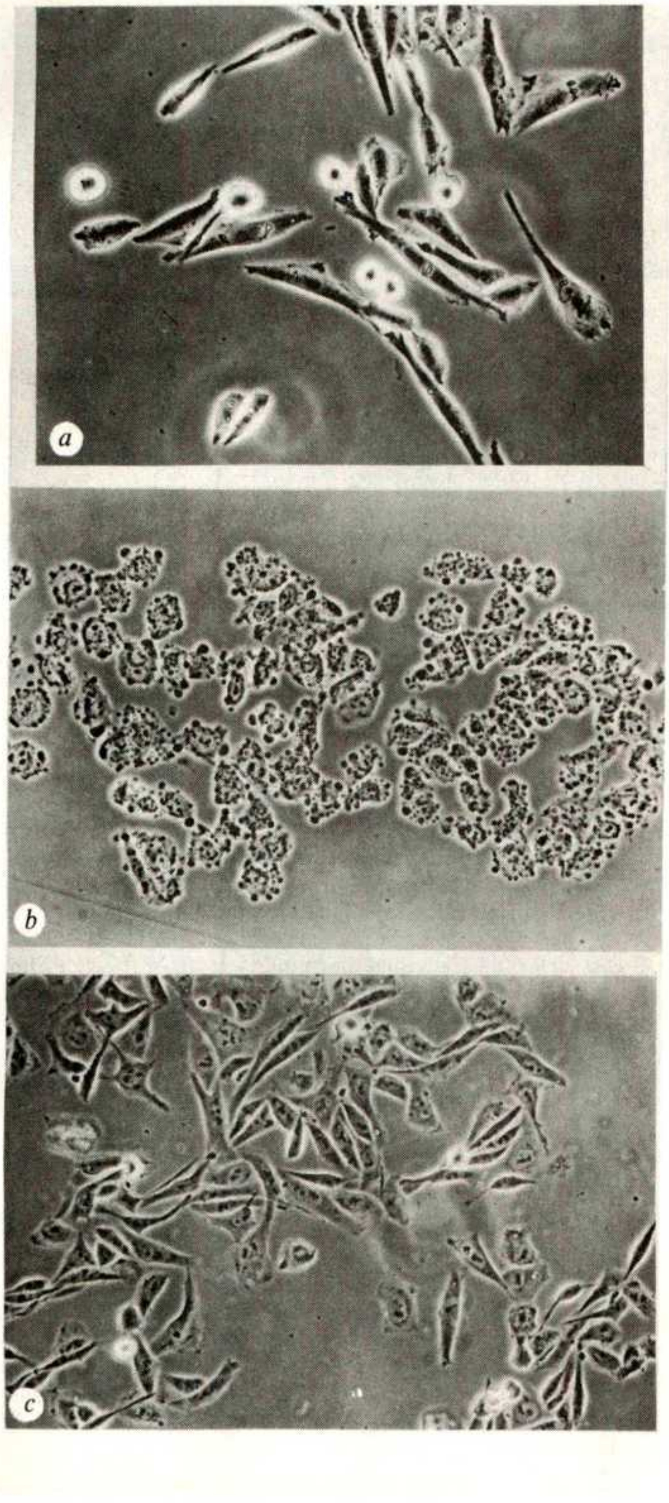
Table 1 Effect of db cyclic AMP on cell shape

Time (h)	0.5 mM db cyclic AMP Epithelial (%)	0.5 mM db cyclic AMP Fibroblast (%)	1.0 mM db cyclic AMP Epithelial (%)	1.0 mM db cyclic AMP Fibroblast (%)
0	93	7	93	7
2	70	30	26	74
4	66	34	20	80
8	35	65	16	84

Coverslip cultures which had been maintained at the non-permissive 33 °C for 48 h were treated with 0.5 mM or 1.0 mM db cyclic AMP. Coverslips were removed at intervals, washed, fixed in 0.1% glutaraldehyde and examined under light microscopy. A minimum of 200 cells was scored for morphology.

curves" were estimated at 39 °C and 33 °C. In this procedure[15], replicate cultures are pulse labelled with ^{3}H-thymidine and the subsequent rate of accumulation of ^{3}H-labelled mitoses is measured, thereby providing an estimate of cell cycle traverse from S to G_2 to M. In spite of an initial low rate at 33 °C, all cells labelled with ^{3}H-thymidine reached mitosis by 10 h (Table 2). These results together with the data in Fig. 1*b* indicate that cs4-D3 is arrested at the G_1 interval. Furthermore, analysis of DNA distribution profiles by flow microfluorometry in a fluorescence activated cell sorter (D. B. T. and D. F. Capellaro, unpublished results) have shown that after 24 h at 33 °C most cells (>75%) had the 2c DNA content characteristic of G_1.

Fig. 2 Morphology of cs4-D3 at the permissive temperature, 39 °C (*a*); at the non-permissive temperature, 33 °C for 48 h (*b*), and at 33 °C for 48 h followed by a 6-h treatment with 1 mM db cyclic AMP (*c*) (see Table 1).



So far no mammalian *cdc* mutant has been synchronised after a temperature block and, in most instances, mutants show loss of viability by 24 h at the non-permissive temperature. In contrast, the cold-labile defect of clone cs4-D3 was reversible at all times tested and cells could be synchronised by a temperature shift (Fig. 3). Cell cultures (100–300 replicate culture vials) in the exponential phase of growth were stepped down to 33 °C for 24 h (Fig. 3*a*) or 48 h (Fig. 3*b*) or 96 h (Fig. 3*c*) or 240 h (Fig. 3*d*) and then returned to 39 °C; samples were monitored at different times for cell number and incorporation of ^{3}H-thymidine into DNA. There was a constant and reproducible lag period followed by a three- to fivefold increase in ^{3}H-thymidine incorporation and a plateau increase in cell number by 30 h (Fig. 3*a*–*c*). Optimum synchrony was obtained after 48 h at 33 °C (or 72 h, data not shown) and, at the first division, increase in cell number was often equal to a theoretical doubling (division index, 0.8–1.0) with respectable synchrony for at least two cell cycles. The length of the prereplicative lag before the onset of DNA synthesis was related to time spent at the non-permissive temperature. After 24 h (Fig. 3*a*) or 48 h (Fig. 3*b*) at 33 °C an 8-h lag preceded the onset of DNA synthesis; this was extended to 12 h in cultures maintained at 33 °C for 240 h (Fig. 3*d*). It should be emphasised that an extended delay of 12 h does not indicate loss of viability or growth potential; autoradiography studies showed that most cells could

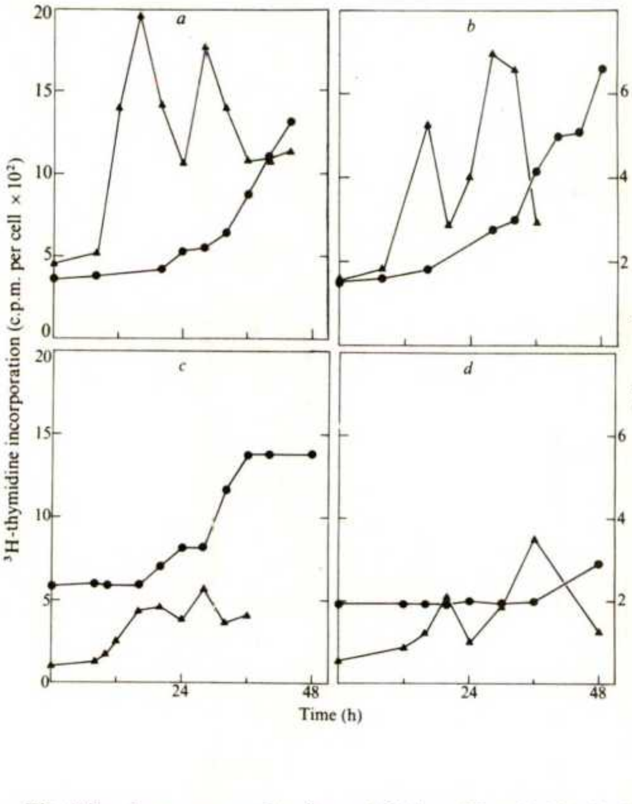


Fig. 3 Synchronous growth, after a shift from 33 to 39 °C, of cell cultures maintained for 24 h (*a*); 48 h (*b*); 96 h (*c*); and 240 h (*d*) at the non-permissive 33 °C. Each experimental group represents a minimum of 100 cultures established in glass scintillation vials. At various times, quintuplicate samples were exposed to 6-^{3}H-thymidine (1 µCi ml^{-1}; 5 Ci mmol^{-1}) for 30 min, washed with EBD medium containing 2 mM thymidine and then ice-cold trichloroacetic acid (10% w/v) and counted directly in a Beckman liquid scintillation counter. Values for cell number represent a mean for three estimations. Cell number (●), ^{3}H-thymidine incorporation into DNA (▲).

Table 2 Estimation of ^{3}H-thymidine-labelled mitoses

Time (h)	Labelled mitoses (%)	
	39 °C	33 °C
0	0	0
2	49	10
3	79	55
4	95	98
5	99	98
6	100	100

Coverslip cultures were established in Leighton tubes (10^4 cells; 2 ml), incubated at 39 °C for 48 h to ensure exponential growth, and then pulse labelled with 6-^{3}H-thymidine (0.2 μCi ml^{-1}; 5 Ci mmol^{-1}) for 30 min. Cells were washed twice with EBD medium and recultured at either 39 or 33 °C. Coverslips were removed at intervals, then fixed in acetic acid–ethanol (1:3; v/v) and autoradiographs were prepared using Ilford nuclear emulsion.

be induced to divide (> 90% at 96 h) after 240 h at 33 °C.

A similar kinetic response has been reported by Augenlicht and Baserga for serum stimulation of quiescent human fibroblasts[16]. The length of the prereplicative phase varied according to the duration of the quiescent period. Initially, a prereplicative period of 8 h was observed before DNA synthesis and this extended to 14 h if cultures were deprived of serum for a further 4 d. Augenlicht and Baserga argued that "cells initially arrest in G_1, subsequently pass into G_0, and continue to go deeper into G_0, as they remain quiescent".

cs4-D3 is remarkable in that, at 33 °C, it expresses the phenotype of normal, differentiated cells—a viable quiescent state. *In vivo* many tissues exist for an indefinite time in a quiescent, G_0 state[17–19], and can be induced to divide by the appropriate stimulus; for example, interaction with antigen for lymphocytes[20] or partial hepatectomy for liver cells[21]. It is doubtful whether the equivalent G_0 state occurs *in vitro*[22,23]. Proliferation of normal fibroblast cultures declines at confluency and cells accumulate at the G_1 (postmitotic) interval of the cell cycle; they can be induced to divide further either by reculture or addition of fresh serum, but stationary cultures lose cloning efficiency after some days. Even this limited period of quiescence is absent on transformation; tumour cells continue to proliferate at low serum concentrations, albeit at a reduced rate, "piling-up" at confluency and eventually detaching from the substratum. This is particularly true for wild-type CHO: cells round up at confluency and detach from the substratum while stationary cultures, induced by isoleucine starvation[24], show rapid loss of cloning efficiency. It might even be argued that G_0 is a feature of the differentiated state, *in vivo*.

We suggest that cs4-D3 reverts at the non-permissive temperature from a neoplastic phenotype of the parental CHO cell to that of a "normal" cell. Phenotypic differences between normal and transformed cells have been described but many were found to be a function of cell division. An important difference between normal and transformed cells might be ability of the former to maintain a viable, quiescent state.

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letters to nature

Search for X-ray emission from Nova Cygni 1975

WE have used the SAS-3 X-ray observatory to search for X rays from Nova Cygni 1975 before, during and after the time of optical maximum (1975 August 30–31). No X rays were detected at any time over the spectral range 0.1–50 keV, and from these negative results we can place very low upper limits on the ratio of X-ray to optical luminosity.

The light curve and spectrum of Nova Cygni 1975 show it to be a fast common nova of spectral type around A2 near maximum. (All optical and infrared data are taken from ref. 1.) Figure 1*a* shows the optical growth and decay of the nova, and Fig. 1*b* the 3σ upper limits we obtained for the X-ray intensity (1.5–15 keV) during three periods spanning the optical maximum. The X-ray measurements were taken with argon-filled proportional counters behind large angle slat collimators centred on the satellite *y* axis. During the observations, the spacecraft was being manoeuvred to bring Nova Cygni 1975 closer to the equatorial plane of the satellite, gradually increasing the effective observing area to the maximum of 154 cm^2 and thus lowering the successive upper limits. The upper limits were computed assuming a flat X-ray spectrum.

Two photographic magnitudes[2], $m_{pv} > 9.6$ on August 28.059 and $m_{pv} = 7.5$ on August 29.052, show that the nova was already brightening rapidly at the time of our first observations, August 28.5–28.9. At the time of the second observations, August 29.9–30.5, the visual magnitude was $m_v = 2.0$, implying an intensity of 5.95×10^{-21} erg cm^{-2} s^{-1} Hz^{-1} at 5,500 Å, which gives $[I_x(\sim 4\ \text{keV})]/I_{op}$ (5,500 Å)] $< 2.8 \times 10^{-8}$. We have converted these intensity ratios to luminosity ratios. With little interstellar reddening[3,4] an A2 star has a bolometric correction of −0.3 (ref. 5) giving $m_{bol} = 1.7$ for August 29.9–30.5 and, thus, $L_{op} \simeq L_{bol} = 5.2 \times 10^{-6}$ erg cm^{-2} s^{-1}. This yields $[L_x(1.5\text{–}15\ \text{keV})]/L_{op} < 1.0 \times 10^{-4}$. Bolometric corrections derived for normal stars may, however, not be applicable to nova shells of similar temperatures. This introduces an uncertainty factor of at least 2 into the optical luminosity.

On September 1.64, repeated scans were made back and forth over the object for several minutes, giving a large increase in sensitivity. In addition, Nova Cygni 1975 was now in the circular fields of view of the thin-window, low energy (LE) proportional counters (20 cm^2, 2.9° FWHM) and the xenon-filled proportional counters (101 cm^2, 1.7° FWHM) so that upper limits are available for energies from 0.1 to 50 keV. These are shown in Fig. 2.

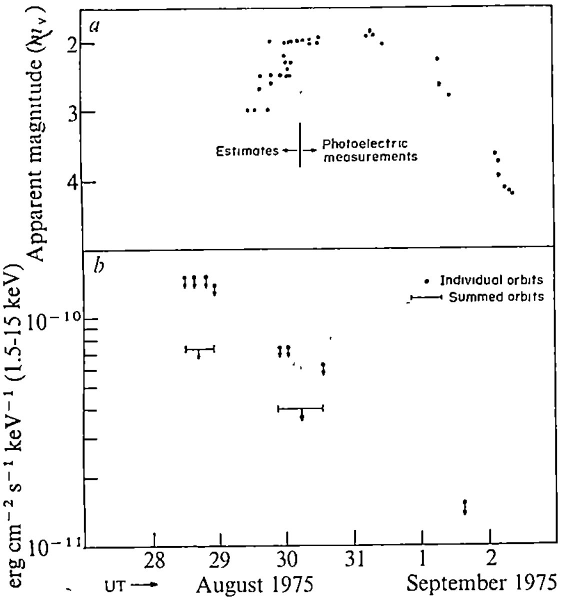


Fig. 1 *a*, Optical light curve for Nova Cygni 1975. *b*, 3σ upper limits on 1.5–15 keV X rays from Nova Cygni 1975 observed by SAS-3 at various times before, during and after optical maximum.

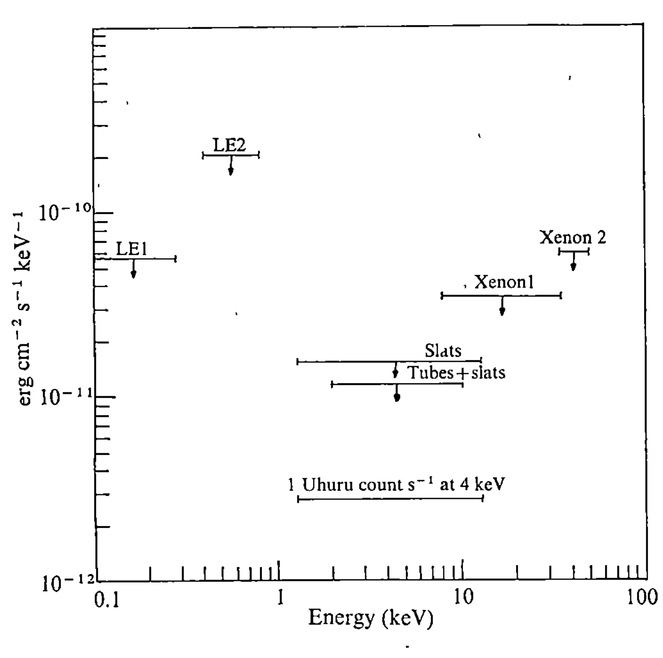
The optical luminosity decreased to $m_v = 3.1$ between our second and third set of observations, when we find $[L_x(1.5\text{–}15\ \text{keV})]/L_{op} < 8.1\times10^{-5}$. In the energy range 0.1–0.3 keV interstellar absorption is important. From the rapid decline after maximum, de Vaucouleurs[6] estimated a distance of ~1.6 kpc. At this distance, even Sco X-1 is essentially invisible at 0.1 keV. Our upper limit of the X-ray energy received at the Earth is

$$[L_x(0.1\text{–}0.3\ \text{keV})]/L_{op} < 5.4\times10^{-6}$$

but this ratio may be higher at the source.

Porton and Clark[7] reported an upper limit of 10 mJy to radio emission at 10.6 GHz near optical maximum. Hjellming[8] reported an upper limit of 4 mJy at 8,085 MHz on September 3, soon after maximum. Three weeks later, at the same frequency, he detected weak emission (6±2 mJy), which had increased to 14 mJy by October 9.

Fig. 2 3σ upper limits to X-ray emission from Nova Cygni 1975 over the spectral range 0.1–50 keV (September 1.64). For comparison, we show the flux level corresponding to 1 Uhuru count per second, adjusted to a 1.5–15-keV energy range.



We continued SAS-3 observations of Nova Cygni 1975 after the optical maximum. The next time that Nova Cygni 1975 was close to the equatorial plane of the satellite was September 20–24, three weeks after optical maximum. No X rays were seen with the following 3σ upper limits (1.5–15 keV)

September 20.46–20.58, 4.4×10^{-11} erg cm^{-2} s^{-1} keV^{-1}

September 22.92, 5.4×10^{-11} erg cm^{-2} s^{-1} keV^{-1}

September 24.9, 4.4×10^{-11} erg cm^{-2} s^{-1} keV^{-1}

The intrinsic properties of Nova Cygni 1975 such as peak luminosity, ejection velocity and mass are typical for common novae (see ref. 3). Because of its high relative optical brightness, we have been able to place a strong upper limit on its X-ray to optical luminosity, $L_x(1.5\text{–}15\ \text{keV})/L_o < 10^{-4}$. For comparison with known X-ray sources, we note that L_x/L_o ranges from 10^{-8} in the quiescent Sun to 10^3 in Sco X-1. Nova Cygni 1975 is the first common optical nova for which a significant X-ray upper limit has been set. What is the significance of this result?

Common optical nova outbursts are thought to result from the explosive burning and ejection of material from a white dwarf which has accreted matter over an extended period from its binary companion star[9,10]. Since the explosion itself is caused by nuclear reactions at the base of a surface layer of material accreted on to the white dwarf, there is no *a priori* reason to believe that significant X-ray emission should directly accompany the explosive event. The spectral types of Nova Cygni 1975 and other common novae near maximum light[11] indicate effective temperatures of $\sim10^4$ K. Such temperatures are entirely consistent with the small upper limit on L_x/L_o that we have obtained.

Brecher and Morrison[12], however, suggested that the subsequent shock heating of any present circumstellar material by the ejected nova shell would give rise to X-ray emission with a flux which depends mainly on the density of the surrounding gas. Wallerstein[13] has also proposed that such a model could account in a natural way for the existence of coronal emission lines seen in novae (which in any case imply the presence of material heated to several million K, a temperature much higher than the colour temperature and absorption line temperature of the nova shell itself). In this picture, only high mass exchange systems (for example, recurrent novae such as WZ Sge, RS Oph, T CrB) should produce X-ray emission at presently detectable levels. If Nova Cygni 1975 is a virgin nova, exploding for the first time (L. Jacchia, private communication), its low mass exchange rate ($dM/dt < 10^{-9}M_\odot$ yr^{-1}) would imply that any associated X-ray emission would be undetectable by present techniques.

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Mass loss in early stages of stellar evolution

IT has been known for some time that stars lose mass between their birth on the main sequence and their death as white dwarfs (or as neutron stars or black holes for more massive stars), and the existence of the solar wind shows that not all the mass loss occurs in a last gasp to form a planetary nebula. Mass loss has also been observed in O–B stars (see refs 1–3), and in red giants[4], but the mass loss rates observed in these stages do not seem sufficient to explain all of the mass loss required for stars to become white dwarfs. The question has always been at what stages and in what manner this excess mass was lost. Suggestions have tended to favour helium flashes for stars with $M < 2.25M_{\odot}$ and possibly double shell instability flashes for stars in the range $2.25M_{\odot} < M < 8M_{\odot}$. Although it is possible that a significant mass is lost during these stages, there is no quantitative support for this speculation, and in particular none for the helium flash. We show here that there is increasing evidence for substantial mass loss during the immediate post-main sequence stages when the star is moving from the main sequence to the giant branch on the Hertzsprung–Russell diagram.

Mass loss at these early stages of evolution may have implications on the subsequent evolution of the star and the amount of nuclearly processed material that is ejected into the interstellar medium. Although possible loopholes can be found in all of the arguments we present, it is interesting to note that the naive interpretation of each of these independent arguments points to the same conclusion.

Mäckel *et al.*[5] have estimated the mass of Arcturus to be in the range $0.1M_{\odot} < M < 0.6M_{\odot}$. Arcturus belongs to a moving group with an age comparable to the oldest galactic clusters[6], and to have evolved to a red giant; its original mass was $1–2M_{\odot}$. Thus it seems that Arcturus has lost a significant amount of mass.

The well known post-main sequence relation between luminosity and mass of the core (hydrogen-depleted interior) can be used to help determine the evolutionary stage of Arcturus. Calculations by Kozlowski and Paczynski[7] show that for a star to have the luminosity of Arcturus, $\log (L/L_{\odot}) = 2.36$, the mass must be $\geqslant 0.3M_{\odot}$ to be moving along the giant branch, and $\geqslant 0.9M_{\odot}$ to be burning its helium core. Similar calculations by Paczynski[8] indicate that a core mass of $\sim 0.52M_{\odot}$ is necessary to give this luminosity if the star is on the asymptotic branch. The luminosity is too low for Arcturus to have begun double shell flashes, and the normal composition, except for the CNO isotopes, indicates that there has been no mixing of the products of helium burning with surface layers as one may expect during double shell flashes. If the mass determined by Mäckel *et al.* is accurate, it seems most likely that Arcturus is on the giant branch and has not yet reached a helium flash point.

The composition may give additional clues as to when Arcturus lost its mass. The $^{12}C/^{13}C$ ratio has been shown[9] to be 7.2 ± 1.5. If the $^{12}C/^{13}C$ ratio was initially solar (90), one would expect that simple deep convective mixing while on the giant branch reduces the $^{12}C/^{13}C$ ratio from 90 to 25. Varying the initial $^{12}C/^{13}C$ ratio to 40 changes the final ratio to 20. No further change in the $^{12}C/^{13}C$ ratio is expected until the star begins double shell flashes, after which the $^{12}C/^{13}C$ ratio rapidly goes to 3.5 (ref. 10). If Arcturus has not yet reached the double flash stage, it is difficult to understand its low $^{12}C/^{13}C$ ratio. Dearborn *et al.*[11] have shown that the $^{12}C/^{13}C$ ratio can be reduced below 10 if half the mass of the star was ejected before material reaching the surface convection zone had been CNO processed. (Even if Mäckel *et al.*'s mass estimate should prove low, it is possible that Arcturus started near $2M_{\odot}$ and now has a mass of $1M_{\odot}$.)

The low $^{12}C/^{13}C$ ratios observed in most K and M giants[12–15] are consistent with these stars losing 20–30% of their mass before the convection zone reaches the ^{13}C-rich layer in the star. Some giants have $^{12}C/^{13}C$ ratios in the range 20–30, but are still consistent with some mass loss (10%) at an early stage of post-main sequence evolution. Arcturus cannot be dismissed as a pathological case. Dziembowski and Kozlowski[16] have shown that AI Velorum stars are theoretically consistent only with hydrogen shell burning stars of masses $0.2–0.25M_{\odot}$. Stars of such low mass will never ignite helium. Kinematic studies of these stars imply progenitors with masses $\sim 1M_{\odot}$. Also the masses of the helium core of stars reaching the giant branch would be larger than those of the AI Velorum stars for $M > 2M_{\odot}$. Since the AI Velorum stars were not able to ignite helium, they must have lost mass immediately after depleting the hydrogen in their cores.

It has been known for many years that to form a white dwarf from a star more massive than $1.4M_{\odot}$, substantial mass must be lost. From the number of white dwarfs in the Hyades and from supernovae statistics, it seems that stars up to at least 3 or $4M_{\odot}$ lose sufficient mass to become white dwarfs. A white dwarf of $\sim 0.7M_{\odot}$ has been observed in the Pleiades (see ref. 17) which have a main sequence turnoff mass of $\sim 6M_{\odot}$. Integration of Reimers' formula[4] for mass loss from a $7M_{\odot}$ star implies a mass loss of only $1.6M_{\odot}$ before carbon detonation (most of this is lost at extremely late stages). Estimates of mass ejected in planetary nebulae[18] do not give sufficient leeway to make up this difference, and a $7M_{\odot}$ star must lose another $5M_{\odot}$ somewhere along its evolutionary track to form a $0.7M_{\odot}$ white dwarf. The lack of success of Reimers' formula in yielding a sufficiently large mass loss does not mean that it does not apply at all to luminous red giants, but simply indicates the need to search for an additional mechanism for losing mass.

If stars lose 20–30% (or even 50%) of their mass when subgiants, there are few directly observable consequences. The luminosity and temperature of most field stars are affected more by unknowns such as age and original mass than mass loss. One would expect, however, to see some effect in the Hertzsprung–Russell diagram of clusters if mass is being lost. First, as was shown by Chiosi and Nassi[19], mass loss on or near the end of the main sequence causes a slightly smaller core to form, and stars approach the giant branch with a lower luminosity. Second, if different amounts of mass are lost from stars in the same clusters, there will be a spread in the horizontal branch or clump (for Pop. I stars with $M < 3M_{\odot}$). Rood[20] has shown that Hertzsprung–Russell diagrams of globular clusters are consistent with a mass loss of $25\pm3\%$. Varying masses on the giant branch also seem consistent with Osborn's observations of the old open clusters, M67 and NGC2420 (refs 21, 22), and both of these effects also appear in the observations of Flower[23]; he observed several young clusters with $7M_{\odot}$ turnoff masses where there seem to be giant stars of mass $5M_{\odot}$. Lindhoff[24,25] reported on such effects in several galactic clusters, but uncertainty in the turnoff mass (or age, ref. 26), or small numbers of stars made interpretation difficult.

Table 1 is a chart which summarises all of the arguments presented and strongly indicates the possibility that mass loss occurs as a subgiant or at the base of the giant branch (before the convection zone reaching the ^{13}C layer).

One should remember that none of the above arguments is conclusive. Other explanations may be found for the scatter in young clusters, $^{12}C/^{13}C$ ratios, and the apparent low mass of AI Velorum stars. The arguments stated here may be just a large collection of false leads.

Table 1 Summary of evidence for subgiant mass loss.

	Main sequence	Subgiant	Giant branch	Helium flash	Horizontal branch	Asymptotic branch	Double shell flashes	Supernovae	Planetary nebula	Total fractions of main sequence mass which is lost
O–B Supergiants	*								X	?
Luminous red giants						*	*		*	10%
Globular clusters	X				X	X	X	X		20–30%
White dwarf in Pleiades				X				X		90%
Arcturus	X	*	*				X	X	X	> 50%
$^{12}C/^{13}C$ several K giants	X	*	*			X	X	X	X	20–30%
AI Velorum stars	X			X	X	X	X	X	X	≈ 70%
NGC 2420	X				X	X	X	X	X	≈ 30%
Scatter on H–R-diagrams	X			X	X	X	X	X	X	?

*Requires mass loss in this stage.
X, either this stage did not occur, or only small amount of mass loss allowed.
Blanks indicate possibility of mass loss at that stage but no strong evidence.

Mass loss at an early stage can reduce the number of carbon detonation supernovae (which seem to produce excessive amounts of iron peak elements) and allow stars to arrive at the double shell flash stage with smaller envelopes, making it easier to produce carbon stars and FG Sag like objects. If, however, stars lose mass as subgiants or low luminosity giants, it is necessary to ask why some low mass stars, such as those in globular clusters, lose only 25% of their mass so that they can still undergo a helium flash, whereas AI Vel stars lose too much mass to even ignite helium (if Dziembowski and Kozlowski's models are correct). This question is, of course, entangled in how the mass is lost.

It is possible to estimate the energy constraints on the mass loss. The power per unit volume in a convection zone from acoustic[27] and gravity[28] waves are

$$P_{\mathrm{acc}} = 38\,\frac{\rho V_c^{8}}{l\,V_s^{5}}$$

$$P_{\mathrm{grav}} = 100\,\frac{\rho V_c^{3}}{l}\left(\frac{l}{H}\right)^{5}$$

where ρ is the density, V_c is the convective velocity, V_s the sound velocity, l the mixing length, and H the pressure scale height. Unfortunately, both powers end up being proportional to l^7—an extremely uncertain parameter.

The acoustic energy flux is sufficient to sustain a tremendous corona and could easily provide the required mass loss to produce the $^{12}C/^{13}C$ ratios. If, however, this is the power source of the solar corona, then only a small fraction of the flux emerges to drive the solar wind. Calculations of the emergent flux have been attempted by Whitaker[29] and DeLoore[30], but are extremely uncertain. There is also no theory explaining the possible effects of metal concentrations, magnetic field or rotation to explain the apparent variation of the mass loss.

Those arguments strongly suggest the possibility of substantial mass loss at an early stage of post-main sequence stellar evolution. Further observations are required for some indication of mass loss, such as of coronae, particularly of stars which have recently left the main sequence and are in the process of moving to the giant branch. They also indicate the need to look in detail for a reasonable mechanism to explain the required mass loss.

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Accretion-disk scenarios of the precursor peak in X-ray transients

ONE of the striking features of a number of the transient X-ray sources is the 'precursor peak', an intensity maximum which precedes the rise to primary maximum and is separated from it by a dip. It is characteristic of A0620−00 (refs 1, 2), A1524−62 (ref. 3), A1118−61 (refs 4, 5), and A0535+26 (refs 6–9). The time scale across the precursor and subsequent dip is ~4–20 d, though for A0620−00 the dip itself spans a time of only ~10 h. Apart from flares, which have a much shorter time scale, there are similar secondary peaks observed during decay for some of the sources—A1524−62 and A0535+26, for instance. Any model of these transients must account for such features along with the concurrent softening of the X-ray spectrum in some cases, the temporal asymmetry of the light curve and, for sources A1118−61 (ref. 5) and A0535+26 (ref. 6), periodicities of 6.755±0.010 min and 104.14±0.16 s, respectively. It is possible to explain qualitatively the precursor and related phenomena on the basis of accretion-disk behaviour with a rising accretion rate $\dot{M}$, followed by its gradual subsidence and the emptying of the disk. In this paper I present three schemes.

I start with two preliminary comments. There are models where a decreasing $\dot{M}$ gradually uncovers the X-ray producing regions buried beneath an optically thick blanket of accreting material[10]. This seems unlikely for a number of reasons (A. P. Willmore, unpublished)—the gradual softening of the spectrum and the skewness of the light curve towards the decay phase.

Second, our reliance on available thin-disk models is, at this stage, not crucial for our work. Except for those of Lightman[11,12], all models are either independent of time[13,14] or time averaged[15], assuming that $\dot{M} = 4\pi rh\Sigma v_r$ is constant; here r is the radius, h the disk half-thickness, $\Sigma = \Sigma(r)$ the surface density and v_r the radial drift velocity. Nevertheless, their use in the present context is rigorously justified as long as the time scale t_M over which $\dot{M}$ varies satisfies[12].

$$t_{\mathrm{M}} > t_{\mathrm{D}} \tag{1}$$

where $t_D \simeq (r/h)^2\,\alpha^{-1}\,t_H$ is the drift time scale, the time it takes Σ to diffuse a distance r. Here α is the 'catch-all' viscosity parameter and t_H is the hydrodynamic time scale; $\alpha \simeq 10^{-3}$–1, $t_H \simeq 10^{-3}$–10^{-2} s. Thus, if $\dot{M}$ is increasing over a period of days, equation (1) will generally be satisfied. Even when it fails, the models probably give us a qualitatively correct picture of the evolution.

In the first scenario, Roche-lobe overflow begins and increases; a disk forms. At first, the very soft X-ray flux from portions of the disk with large radii will dominate. But eventually, as a stable type II (electron-scattering opacity and matter dominated) inner region develops, the luminosity will become significant in the 1–20-keV range, where the Ariel V transients have been detected. The continuing rise in $\dot{M}$ will lead to increasing luminosity, since the flux $F \sim \dot{M}$ throughout the disk[13,14].

For the two sources A1524−62 (ref. 3) and A0620−00 (ref. 2) a significant spectral softening is evident during the luminosity increase, but at different phases of the light curve. In the former it occurs during the rise to the precursor peak; for A0620−00, however, the spectrum remains relatively hard across the precursor, with the softening occurring only afterwards—during the rise to principal maximum. How is this to be interpreted?

The intensity dip is caused, in this scheme, by disk thickening, primarily in the cooler outer regions, which absorb some of the radiation originally destined for the observer. Some of the harder photons from the inner regions of the disk and/or from the surface of the compact object, are reprocessed and re-emitted at lower energies (Cunningham, C. T., unpublished). This mechanism can account for the spectral softening, which in A1524−62 is in proper correspondence with the intensity dip for this picture: thickening (and spectral softening) occurs during the precursor peaking and then reaches a point where the outer region of the disk begins to intercept radiation directed towards the observer. For A0620−00, however, this correspondence fails—and it seems impossible to apply the scenario properly

The absence of spectral softening (as in A1118−61 (ref. 5)) does not necessarily rule out this scheme. Whether it will be observed in a given case depends on the observer's angle of inclination and other factors and can be predicted only on the basis of a detailed quantitative model.

The gradual thickening postulated here would arise primarily from the reabsorption of radiation itself—from the hotter inner regions of the disk and from the surface of the compact object. As the disk thickens, of course, it becomes even more susceptible to such absorption and reheating. Thus it is possible that through this process the outer regions would swell into a cloud shrouding the entire compact object and inner-disk system.

The continuing increase in $\dot{M}$ forces the luminosity of the thick disk or cloud configuration from the dip to the primary maximum. Then, as $\dot{M}$ begins to decrease, the transient enters its decay phase, which has a time scale of months. It is likely that this is just the emptying time t_e of the disk modulated by $\dot{M}$. From both related observational (Bailey, J., unpublished) and theoretical[16] considerations, t_e, for reasonable values of $M/M_{\odot}$, binary period and disk radius, is of the required order. If an extensive quasi-spherical cloud shrouds the disk, its accretion time may also be important. A secondary maximum during decay, such as is seen in A0535+26, can be accounted for in terms of thick disk subsidence to a thin disk.

For sources like A1118−61 and A0535+26, it is important to establish whether their periodic components persist at the same level through the peak and dip and on into the principal maximum, or are characteristic only of certain stages of the transient's evolution. Such information would also help determine whether the precursor phenomenon arises primarily from disk behaviour or from mechanisms associated with, say, funnel accretion on to the polar caps of a neutron star. Though these observed periodicities are not directly explained by the disk behaviour itself in this scheme, they are not inconsistent with it. They may be a characteristic, for example, of the component of radiation emanating from the polar caps of the neutron star in a neutron-star thick-disk system.

The second scheme pertains to non-magnetic situations, black holes or unmagnetised neutron stars, and applies primarily to A0620−00. It begins and ends like the first. But, in the course of the rise to the precursor peak with increasing $\dot{M}$, either an unstable radiation dominated inner region appears[11,12] or thermal instabilities[18] develop from the severe density thinning across the inner edge (W.R.S., unpublished). In either case the inner region of the disk will blow up into a very hot, essentially spherical cloud, and inner disk models like those of Thorne and Price[19], Lightman and Shapiro[20], Eardley *et al.*[21], and Shapiro *et al.*[22] will be applicable. The onset of such instabilities occurs at the precursor peak, and the subsequent dip is formed as the luminosity contributed to the 1–20-keV range by the hotter inner region is lost. The continually increasing $\dot{M}$, however, quickly overcomes the dip and moves the transient towards its maximum.

This sequence would explain why the spectrum remains hard during precursor peaking in a source like A0620−00 and begins softening only afterwards. It would also account for the relatively sudden dip with recovery on a time scale of hours. A crucial test of the model would be significant spectral softening in the narrow dip itself.

It is possible that, corresponding to the occurrence of the dip, hard X-ray emission in the 100–500 keV range from the very hot inner cloud would make its appearance. But, then again, such a source might might not be evident, particularly if this cloud is optically thin. For then the flux $F \sim n^2T^{\frac{1}{2}}$ instead of the much more substantial $F \simeq bT^4$ of black-body radiation. The swollen inner region, however, might still function to boost soft photons into medium hard X-ray range by inverse Compton scattering[21,22]. G. F. Carpenter, M. J. Coe, A. R. Engel and J. J. Quenby (unpublished), in fact, have shown that the spectrum of A0620−00 is similar in many respects to that of Cygnus X-1, where just such a model closely fits the data[21,22].

The third scenario applies only to a black hole in a stellar-wind environment and demands both an increasing $\dot{M}$ and an increasing specific angular momentum L of the accreting material. Modulation of L could be provided by a highly eccentric binary orbit, but this seems somewhat questionable on other grounds[23]. The general picture involves the formation at very small radii of an initial marginal accretion disk[24,25] ($r=4M$ to $r=12M$ in the Schwarzschild case—in units where $G=c=1$), which grows into a stable disk as $\dot{M}$ and L increase.

We can describe the disk evolution in terms of the orbital topography of the Schwarzschild metric[26]. The specific energy (including rest mass) of the infalling material is $E \simeq 1$. As L approaches and then becomes larger than $L_{\rm ms} \simeq 3.464M$(the specific angular momentum for a marginally stable circular orbit at $r_{\rm ms} \equiv 6M$), a chaotic transitional disk will form, heat up and radiate[25]. Such a disk will extend outwards, not from $r_{\rm ms}$, but from $r_{\rm mb} = 4M$ (the marginally bound circular orbit). Since the material has both a large E and $L < 4M$, a large proportion of it will be in unstable circular and elliptical, and stable elliptical, orbits extending from $r_{\rm mb}$ outwards ($E_{\rm mb} = 1$ and $L_{\rm mb} = 4M$). As the material loses some of its energy and angular momentum, a large proportion of that between $4M<r<6M$ will first migrate into stable circular orbits at $r>r_{\rm ms}$ before spiralling on to the secondary.

As L and $\dot{M}$ increase, the disk grows radially, and the inner region between $4M$ and $6M$ disappears, superseded by a stable 'classically' described thin disk with inner edge at $r_{\rm ms}$. The formation of the chaotic inner disk would explain the initially hard spectrum, the precursor peak would arise from the gradual build up of this inner disk with increasing $\dot{M}$ and L, and the dip, along with some of the spectral softening during the rise to primary maximum (as in A0620−00), to the dissipation of the transitional disk as the stable outer disk developed. The rise to primary maximum and the continuing softening of the spectrum would then be the result of both the gradual growth of the stable disk and the increasing $\dot{M}$. The time scale across the precursor peak will depend on how long accretion with a marginal L continues, and that across the dip on the time it takes for the rising accretion rate to recover the ground lost through dissipation of the chaotic disk—for A0620−00 this would have to be ~ 10 h. The time scale of decay, again, is just $t_{\rm e}$ modulated by $\dot{M}$. A marginal-disk stage should be a briefly discernible feature during the decline.

I hope this work will open theoretical avenues of approach to the precursor phenomenon and motivate further investigation of this unexpected feature.

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A return to the pre-1971 intensity level and a 5.6-d modulation for Cyg X-1

CYG X-1 exhibited three pronounced X-ray intensity increases during 1975. The last of these, unlike the previous two, has not rapidly decayed back to its pre-increase value. Such a persistently high emission level from Cyg X-1 has not been observed since the source 'transition' in 1971[1].

A 5.6-d modulation synchronised with HDE226868 was observed over the first six months of the Ariel V all-sky monitor (ASM) data accumulation before the April 1975 increase[2], but was not detectable immediately afterwards. We report here the detection of a similar 5.6-d modulation in the 'high-state' data obtained over the 3-month interval November 1975–January 1976.

The Ariel V ASM experiment has monitored Cyg X-1 quasi-continuously since its launch in October 1974. No flare-like increases were observed until April 1975, when the source suddenly increased to an intensity level in excess of that of the Crab Nebula. The rise[3], maximum[4] and decay[5] were marked by considerably more variability on time scales of hours, or more than was the relatively quiescent pre-increase temporal record. A smaller increase was reported in September[6], which displayed the same variability and decay time of ~ 1 week. The third increase in November[7,8] was to the level of the April event, displaying the same variability. It did not, however, decay over the same time scale[9], and has shown no sign of decay after 3 months. Figure 1 displays the daily averages of the Cyg X-1 data accumulated between launch and January 31, 1976 by the ASM.

The folded data from both the six months before the April 1975 flare and the 3-month interval November 1975–January 1976 are displayed in Fig. 2, where we have used the ephemeris of ref. 10 to define the epoch of superior conjunction. The folds are in five bins, with the ordinate for each bin defined as the source intensity relative to the average of the four bins excluding superior conjunction. This average value for the new data is more than twice that of the pre-April 1975 measurements.

The light curve decrement (that fraction of the total source emission over one 5.6-d cycle which is deficient at superior conjunction) is 0.019 ± 0.003 for the lower trace of Fig. 2, compared with 0.029 ± 0.004 for the upper trace. This level of modulation was not detectable in the ASM data between the April and November flares, which may have been the result of uncorrelated source intensity variations masking the effect (the experimental value of the decrement was 0.008 ± 0.009 during this interval). Daily averages for the April, September and November–January increases are displayed in Fig. 3, combined with a 5.6-d grid from the ephemeris of ref. 10. It is obvious that the uncorrelated intensity variations prevent the direct observation of the reported level of 5.6-d modulation over individual cycles in general, and the effect can only be demonstrated convincingly by averaging over many cycles.

In ref. 2 it was suggested that the modulation observed before the April flare might be associated with the build up to the increase, and might be observable only just before such increases. The new results reported here suggest that this level of X-ray modulation may be generally character-

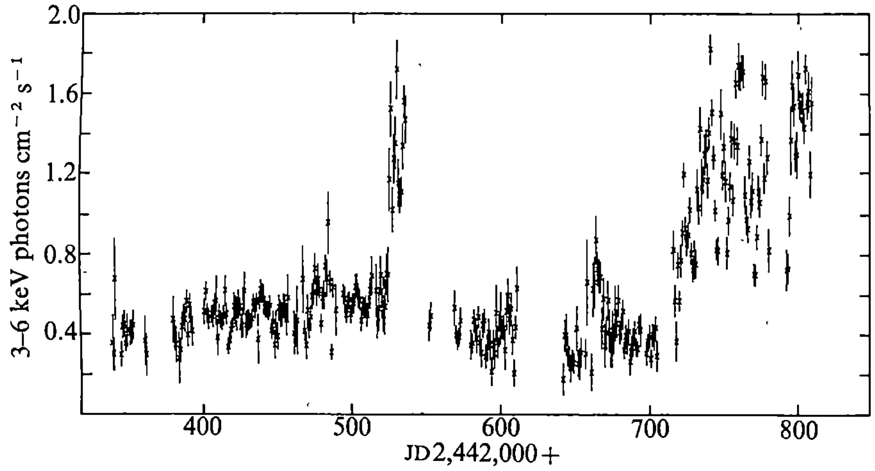
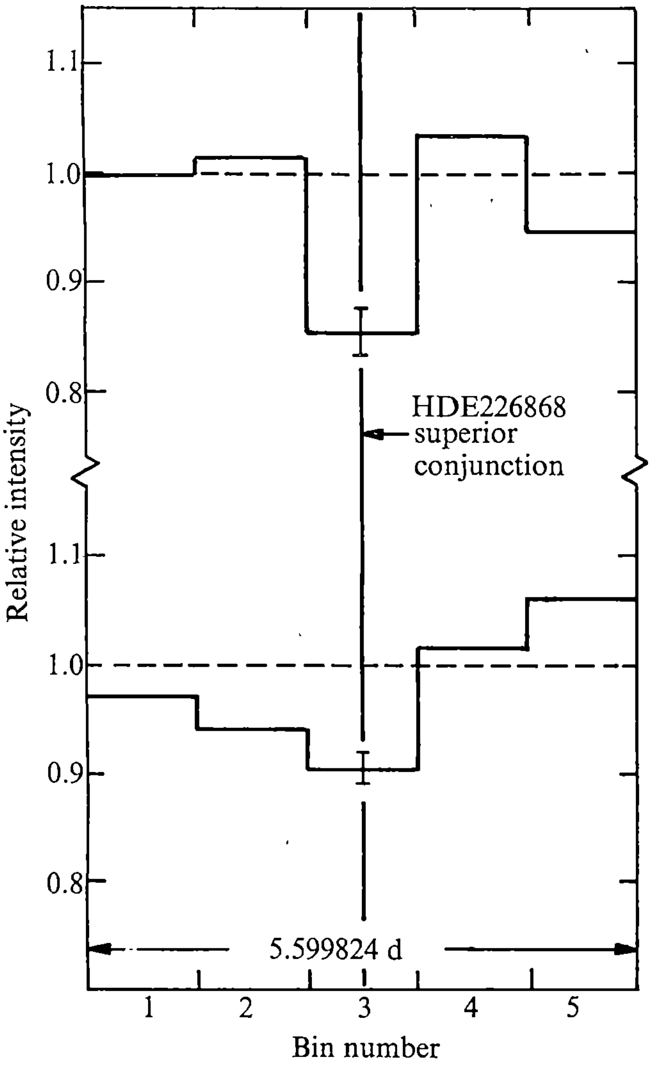


Fig. 1 Daily averages for more than one year of ASM measurements of Cyg X-1. The ordinate is natural for the experiment, with the Crab Nebula giving ~1.2 in the same units.

istic of Cyg X-1. The orbital analyses of Hutchings (see ref. 11) and Bolton[10] suggest that an inclination i of ~30° is necessary to reconcile the optical measurements, in contrast to the i ~60° required if the present results represent an eclipse of the source by a completely-filled primary Roche lobe. It would seem, therefore, that our results are most easily explained by scattering from ionised material between the two stellar components which extends outside the orbital plane (see ref. 10). The material must be largely ionised because a much larger effect has not been reported at lower energies. As described in ref. 9, the magnitude of the present level of modulation is approximately an order of magnitude in excess of the expectation from the more sharply defined 'absorption dips' which have been attributed to the effect of neutral material, so that it is not at all clear how these two classes of superior conjunction decrements are related. In any case, the decrement at > 3 keV reported here appears to be consistent with a stable few per cent underlying average periodicity in both the high and low intensity states of Cyg X-1. We cannot ascertain unambiguously whether this effect is present every cycle at the average level, or whether the effect is larger and present only a fraction of the time. Figure 3 does contain a few pronounced minima which are synchronised with superior conjunction, but it also contains many which are not. The final determination of this question must await a detailed analysis of many high state cycles (of which only ~15 are

Fig. 2 Data taken before the April 1975 increase (upper trace, November 1974–April 1975) and after the start of the November 1975 increase (lower trace; November 1975–January 1976) folded in 5 bins using the HDE226868 ephemeris[10].



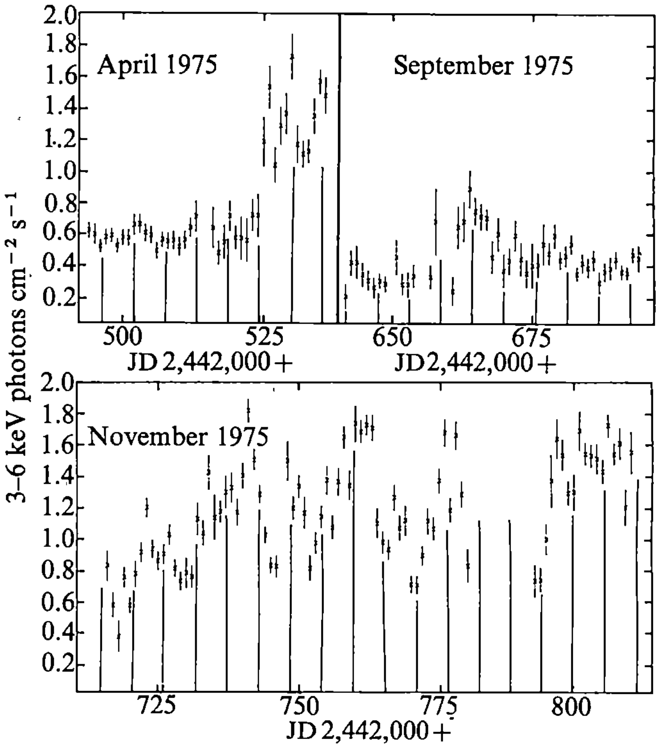


Fig. 3 Daily averages at the times of the three 1975 increases, with a superposed HDE226868 superior conjunction grid. On a finer time scale, the asynchronous intensity variations are even larger than those reflected in the plotted daily averages.

included here). Should the intensity remain high indefinitely, the ASM should be capable of resolving the issue eventually, as it is expected to continue operation for at least another year.

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Note added in proof: Shortly after this manuscript was submitted, Cyg X-1 was observed by the ASM to return sharply to its "low" state[12]. Its average intensity for the week ending on JD 2,442,822 was 1.85±0 04, after which it dropped by a factor of three in one week. The intensity has since been roughly constant at ~0.5 to JD 2,442,851.

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Molecular hydrogen formation on interstellar dust grains

EARLY estimates of the maximum temperature, T_M, of interstellar dust grains (IDGs) consistent with efficient H_2 formation on their surface, yielded values near 10 K (refs 1–3). The expression for T_M is

$$T_M = E_H/\ln\left[\frac{1}{Svn_H A\tau_0}\right] \simeq 10\,\mathrm{K} \qquad (1)$$

where E_H is the binding energy of hydrogen to the dust grain $\simeq$ 400 K, S is the sticking coefficient of H on the grain $\simeq$ 0.5, v is the average velocity of H atoms in the dust clouds $\simeq$ 10^5 cm s^{-1}, n_H is the hydrogen density in the cloud $\simeq$ 10^2 cm^{-3}, A is the surface area of a dust grain $\simeq$ 10^{-9} cm^2, and $\tau_0 \simeq 6\times10^{-13}$ s. Observations have shown this T_M to be unacceptably low (see, for example, refs 5 and 4), and have led to proposals of new or revised mechanisms for interstellar H_2 formation[6–9]. Here we point out that for a certain class of gas–solid systems the characteristic desorption lifetime, τ_0, is many orders of magnitude longer than that used in refs 1–3. For grains made of these materials the original "physisorption model"[1–3] will work for grain temperatures up to 30 K.

The τ_0 of 6×10^{-13} s, used in refs 1–3, comes from the Frenkel theory of adsorption[10], in which detailed balance, single particle partition functions, and equilibrium between the gaseous and adsorbed phases are assumed. These assumptions are not necessarily valid in interstellar clouds. A dynamical theory of desorption makes none of the aforementioned assumptions. Ying and Bendow[11] have formulated such a theory by considering phonon-induced desorption. In particular, they performed detailed calculations for the model system, Ne adsorbed on Xe-covered graphite, and found that τ_0 exceeded 10^{-5} s for temperatures below 40 K. This occurs because the low temperature excitation spectrum of the substrate (Xe on graphite) contains few phonons with energies exceeding the binding energy of the adsorbed atoms. Thus multiphonon processes, which are slow when the phonon gas is dilute, are necessary for desorption. A similar situation does not occur for H adsorbed on the graphite basal plane—a commonly proposed form of IDG. The binding energy of H on graphite, E_H, is about 400 K (ref. 12), while the transverse and longitudinal Debye temperatures are T_t = 760 K and T_l = 2,700 K (ref. 13), respectively. Desorption induced by a single phonon can thus occur and a short τ_0 is the result. This can be seen from the following derivation. The flux of phonons, each with sufficient surface normal energy to cause desorption, times the phonon–adatom cross section, gives the desorption rate, R,

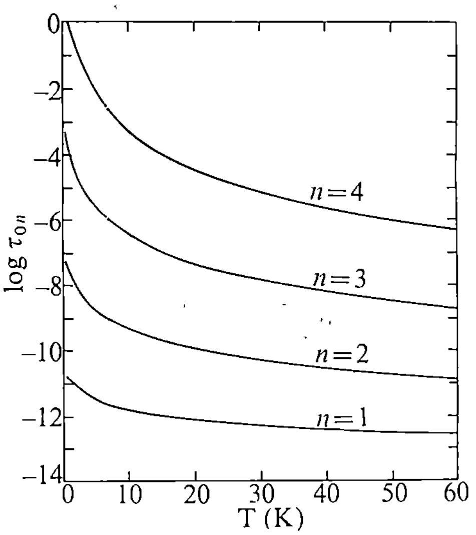

Fig. 1 Log τ_{0n} as a function of T for n = 1,2,3 and 4. τ_{0n} is related to the average lifetime with respect to desorption, τ, by the Frenkel formula[10], $\tau_{0n} = \tau\exp(-E/kT)$, where E is the binding energy of the adsorbed atom and T is the temperature of the substrate.

$$R = \int_{\omega_0}^{kT_D/\hbar}\int_{\theta,\psi} cg(\omega)\sigma(\omega,\theta)\cos^2\theta\,\sin\theta\,d\theta\,d\Psi\,d\omega \qquad (2)$$

where $\omega_0 = E/\hbar$, E is the binding energy of the adsorbed species to the substrate, T_D is the Debye temperature of the substrate (all polarisations are treated equivalently), c is the speed of sound in the substrate, $\sigma(\omega,\theta)$ is the cross section for maximum energy transfer between the phonon and the adsorbed atom $\lesssim \pi(\lambda+d)^2$, $\lambda(\omega)$ is the phonon wavelength, d is the adsorbed atom scale size, $g(\omega)$ is the phonon distribution function in the substrate[14]

$$(g)\omega \simeq V(3/2\pi^2c^3)\{\omega^2/[\exp(\hbar\omega/kT)-1]\}\ \mathrm{s\ for}\ \frac{\hbar\omega}{k} \leqslant T_D$$

and

$$g(\omega) = 0\ \mathrm{for}\ \frac{\hbar\omega}{k} > T_D$$

For the hydrogen–graphite system, $E = E_H \simeq 400$ K, $c \simeq 10^6$ cm s^{-1}, $T_D \simeq 1{,}500$ K, and $d \simeq 3\times10^{-8}$ cm.

Using the facts that $kT \ll E_H < kT_D$ and $\lambda_0 \lesssim d$, equation (2) can be integrated

$$R \lesssim \frac{d^2V}{2\pi^2c^2}\frac{kT}{\hbar}\omega_0^2\exp(-\hbar\omega_0/kT) \qquad (3)$$

This gives a desorption time of the Frenkel form and magnitude,

$$V/R \leqslant \tau_1 = \tau_{01}\exp(\hbar\omega_0/kT) = \tau_{01}\exp(E_H/kT) \qquad (4)$$

where

$$\tau_{01} = \frac{2\pi^2c^2}{d^2}\frac{\hbar}{kT}\frac{1}{\omega_0^2} \simeq 6.0\times10^{-13}\ \mathrm{s}\ (\mathrm{at}\ T{=}100\ \mathrm{K})$$

The inequality in equations (3) and (4) comes from the un-

Table 1 Characteristic desorption times of certain adsorbed gas–substrate systems

Adsorbed species	Substrate	Temperature (K)	Binding energy (K)	τ_0 (s)	Reference
H_2	H_2 covered Cu	1–4	96	4×10^{-9}	23
H_2	CO covered Cu-Ni	10–25		10^{-7}	19
He	He covered Cu-Ni	4–30	30–90	1.5×10^{-7}	21
CO_2	SiO_2	80–130		5×10^{-4}	20
H_2 κ state	CO covered W	300	3,700	1.25×10^{-3}	18
CO	CO covered Co	300–1,000	2,000	2×10^{-3}	22
CO	CO covered Ni	300–1,000	2,000	4×10^{-3}	22
Ar	SiO_2	80–130	250–500	1	20

certainty in the cross section. Specifically, $\sigma(\omega,\theta)$ depends on the phonon polarisation, the availability of final phonon and adatom states, the scattering matrix, and the constraints of energy and momentum conservation.

Graphite is an oddity in that it has a very high Debye temperature. "Softer" materials of low atomic mass constituents such as α-iron, ice, silica or solid carbon monoxide, have Debye temperatures below 200 K (refs 15 and 16). For hydrogen physisorbed on these materials ($E_H \geq 400$ K) two or more phonon impacts are necessary for desorption. For two-phonon desorption to occur, a second phonon must transfer sufficient energy to a hydrogen atom which has been raised to an excited (surface normal) vibrational state by a previous phonon. The second phonon must impact on the excited hydrogen with proper phase and within a time $t \simeq 2\pi/\omega_0 \sim 1.2\times10^{-13}$ s, or else the excitation may decay away by spontaneous phonon emission. The time between successive impacts by energetic phonons is τ_1 (ω), equation (4). Accordingly, the probability that an excited atom will be desorbed by the impact of a second phonon is

$$P(\omega) \simeq \frac{1.2\times10^{-13}\gamma}{[\tau_1(\omega)+1.2\times10^{-13}]} \qquad (5)$$

and the desorption time (at low temperatures) is

$$\tau_2 \simeq \frac{\tau_1(\omega')}{P(\omega)} = \frac{1}{1.2\times10^{-13}\gamma}\left[\frac{2\pi^2c^2}{d^2}\right]^2 \left[\frac{\hbar}{kT}\right]^2 \frac{1}{\omega^2}\frac{1}{\omega'^2} \exp[\hbar(\omega+\omega')/kT] \qquad (6)$$

where γ is a phase coherence factor for the two phonons $\simeq 0.5$ (this corresponds to a $\pi/3$ phase shift between phonons, thus a 50% decrease in energy due to interference effects), $c \simeq 5\times10^5$ cm s^{-1} for iron, ice, silica, and so on, $\hbar\omega$ and $\hbar\omega'$ are the energies of the two phonons, and $\hbar(\omega+\omega') \geq \hbar\omega_0$, the binding energy of the adsorbed hydrogen. The minimum τ_2 occurs for

$$\omega = \omega' = \omega_0/2 \qquad (7)$$

that is, the maximum rate of two phonon desorption occurs when the phonons have equal energy.

This can be generalised to n phonon processes. For n phonon-induced desorption, τ_n is given by

$$\tau_n = \left[\frac{1}{1.2\times10^{-13}\gamma}\right]^{n-1} \left[\frac{2\pi^2c^2}{d^2}\right]^n \left[\frac{\hbar}{kT}\right]^n \frac{1}{(\omega_0/n)^{2n}} \exp(E_H/kT)$$

$$\simeq (n)^{2n}\left(\frac{200}{T}\right)^{n-1} \frac{1.5\times10^{-11}}{T} \exp(E_H/kT)\,\text{s} \qquad (8)$$

which defines a characteristic desorption time

$$\tau_{0n} = (n)^{2n}\left(\frac{200}{T}\right)^{n-1} \frac{1.5\times10^{-11}}{T}\,\text{s} \qquad (9)$$

A graph of τ_{0n} as a function of temperature for n = 1,2,3 and 4 is shown in Fig. 1. It must be stressed that these are lower limits for τ_{0n} because of the uncertainty in $\sigma(\omega,\theta)$. For a four-phonon process, as would be necessary for H desorption from solid CO or to covered surfaces, τ_{04} is 10^{-5} s and T_M (equation (1)) is raised to 30 K.

Long τ_0s have been measured in many experiments. As early as 1928, Cockcroft[17] measured τ_0 of 10^{-9} s at $T = 150$ K. Later experiments showed τ_0 values up to 1 s and several notable examples are listed in Table 1. As can be seen, τ_0 for H_2 on CO covered W[18], for H_2 on CO covered Ni[19] and for CO_2 and Ar on SiO_2 (ref. 20) are greater than 10^{-7} s.

In summary, a dynamical theory of phonon-induced desorption is presented. It is shown that the characteristic desorption time, τ_0, is long at low temperatures if the binding energy of the adsorbed atoms exceeds the Debye temperature of the substrate. This fact has immediate application to the problem of abundant H_2 present in interstellar dust clouds by raising the maximum temperature for efficient H_2 formation to 30 K. From this model we conclude that the basal planes of clean graphite flakes are not effective substrates of H_2 formation, whereas solid CO, H_2O or SiO_2 surfaces are.

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Isotopic fractionation in meteoritic cadmium

ISOTOPE and elemental abundance studies of Cd in this laboratory have led to the discovery of Cd with an isotopic composition which differs significantly from that normally observed in nature. This isotope abundance study was initiated with the primary aim of detecting and measuring neutron irradiation effects in Cd (^{113}Cd (n,γ) ^{114}Cd, σ = 20,000 barn) of similar origin to that observed in Gd (ref. 1), and included a survey of

Table 1 Measured isotopic ratios for cadmium in Brownfield

Date of analysis	Sample‡ identification	No. of data sets	Sample† size (ng)	106/112*	108/112	110/112	111/112	113/112	114/112	116/112
May 22, 1974	AML.H15.81	15	389	0.05371 (0.00010) −8.7‰	0.03800 (0.00009) −1.3‰	0.52337 (0.00049) −4.0‰	0.53381 (0.00049) −1.5‰	0.50398 (0.00046) +2.3‰	1.17797 (0.00098) +3.9‰	0.30462 (0.00034) +10.5‰
July 17, 1974	AML.H15.81	24	253	0.05358 (0.00010) −11.1‰	0.03774 (0.00006) −8.2‰	0.52405 (0.00025) −2.7‰	0.53338 (0.00042) −2.3‰	0.50350 (0.00042) +1.4‰	1.17863 (0.00096) +4.4‰	0.30398 (0.00030) +8.4‰
September 29, 1974	AML.H15.81	13	436	0.05335 (0.00004) +15.3‰	0.03770 (0.00004) −9.2‰	0.52319 (0.00018) −4.3‰	0.53375 (0.00018) −1.6‰	0.50430 (0.00018) +3.0‰	1.17891 (0.00045) +4.7‰	0.30446 (0.00016) +10.0‰
April 18, 1975	AML.H15.117	14	178	nm	nm	0.52363 (0.00040) −3.5‰	0.53430 (0.00042) −0.6‰	0.50463 (0.00048) +3.6‰	1.18077 (0.0015) +6.3‰	0.30449 (0.00030) +10.1‰
September 6, 1975	AML.H15.104	31	261	nm	nm	0.52306 (0.00045) −4.5‰	0.53368 (0.00048) −1.7‰	0.50395 (0.00044) +2.3‰	1.17761 (0.00081) +3.6‰	0.30423 (0.00035) +9.2‰
September 11, 1975	AML.H15.81	4	170	nm	nm	0.52305 (0.00057) −4.6‰	0.53350 (0.00027) −2.1‰	0.50389 (0.00060) +2.2‰	1.17870 (0.00085) +4.5‰	0.30418 (0.00054) +9.1‰
December 4, 1975	AML.H15.117	13	392	nm	nm	0.52305 (0.00020) −4.6‰	0.53357 (0.00021) −1.9‰	0.50415 (0.00023) +2.7‰	1.17887 (0.00033) +4.6‰	0.30453 (0.00015) +10.2‰
		Mean ratios		0.05354 −11.7‰	0.03781 −6.2‰	0.52334 −4.0‰	0.53371 −1.7‰	0.50406 +2.5‰	1.17878 +4.6‰	0.30436 +9.6‰
December 5, 1975	Dilution with normal Cd (2 μg)	18	330 ng +2 μg	nm	nm	0.52514 0.00016 −0.6‰	0.53480 0.00018 +0.4‰	0.50340 0.00019 +1.2‰	1.17442 0.00034 +0.9‰	0.30213 0.00016 +2.3‰
		Laboratory standard ratio		0.05418	0.03805	0.52545	0.53461	0.50280	1.17342	0.30145

*The ratios are not corrected for instrumental discrimination. The error associated with each measurement (in parentheses) is two standard errors of the mean of sets of 10 ratios. The — difference between each isotope ratio and that for laboratory standard is given below the error, where — difference is defined for the 116/112 isotopic ratio as

$$[1-(116/112)\text{sample}/(116/112)\text{standard}]\times 1{,}000$$

The "laboratory standard" ratios are based on measurements on Cd from Cd-rich terrestrial minerals as well as repeated measurements of laboratory reagent Cd (Johnson Matthey Spec-pure metal)

†These figures are calculated from a knowledge of the weight of meteorite used and the cadmium concentration. No allowance has been made for the efficiency of the chemical extraction procedure which is ~ 50%. The chemistry blank for the separation is ≃ 3 ng. In the dilution experiment 2 μg of reagent cadmium was added to the meteorite sample before dissolution.

‡AML, American Meteorite Laboratory, Denver, Colorado.

nm, No measurement made

Cd isotope abundance in terrestrial samples[2] and a range of meteorites. The meteorite data will be reported in detail elsewhere. The Brownfield H3 chondrite, included in the survey because of its enhanced Cd content[3], was found to contain anomalous Cd; some results are given in Tables 1 and 2.

Table 1 shows the Cd to be consistently isotopically fractionated relative to the laboratory standard, with an enrichment in the heavier isotopes. Figure 1 shows that a linear trend exists when the ‰ differences between the mean of the Brownfield data and the laboratory standard are plotted. The fractionation decreased by the expected amount when the meteorite Cd was diluted with reagent Cd (see Fig. 1).

Two samples of Brownfield were double spiked[2] with a ^{106}Cd–^{111}Cd tracer. The ratios measured for the mixtures as well as other data needed for the calculation of the fractionation are given in Table 2. Both mixtures yielded a fractionation of 2.3‰ per mass unit relative to laboratory reagent Cd (Johnson Matthey Spec-pure metal). Measurements made in this laboratory[2] have shown that the reagent Cd used for the experiment has the same isotopic composition as Cd from Cd-rich terrestrial minerals. There is excellent agreement between the double-spike result and the unspiked measurements (Fig. 1).

The data presented here so far show that recognised sources of isotopic discrimination such as chemical processing and mass spectrometry could not be the cause of the observed isotope fractionation. The fractionated Cd could still have a terrestrial origin, however, since Brownfield is a "find"[4]. To establish that the anomalous Cd was most probably present in Brownfield when it fell, it was necessary to find isotopically similar Cd in a meteorite known to be a "fall". A sample of the H3 chondrite Tieschitz was obtained for this purpose and the results of the analyses are given in Table 3. The Cd in Tieschitz is also clearly fractionated, although the results of the two measurements differ.

Further double-spike measurements indicate that both the fractionation and the Cd concentration are extremely variable

Table 2 Double-spiked data for Brownfield

Sample	No. of data sets	106/111*	110/111	116/111	Fractionation relative to: double spike	reagent Cd
					(‰ per mass unit)	
DS Brownfield	15	0.28183 (0.00029)	0.32864 (0.00021)	0.18720 (0.00019)	+1.3	2.3
DS Brownfield	33	0.26880 (0.00019)	0.37558 (0.00022)	0.21484 (0.00014)	+1.3	2.3
DS laboratory reagent	25	0.31491 (0.00039)	0.21237 (0.00022)	0.11684 (0.00010)	−1.1	
DS laboratory reagent	22	0.27180 (0.00025)	0.36802 (0.00016)	0.20736 (0.00024)	−1.0	
Double spike (DS)		0.36985 (0.00042)	0.02294 (0.00006)	0.00672 (0.00003)		

*See Table 1.

Table 3 Isotopic data for Tieschitz†

Unspiked	110/112*	111/112	113/112	114/112	116/112
No. of data sets = 9 Sample size = 50 ng Classification H3	0.52321 (0.00080) −4.3‰	0.5335 (0.0011) −2.1‰	0.5038 (0.0014) +2.0‰	1.1785 (0.0021) +4 3‰	0.30532‡ (0.00080) +12 8‰
Double spiked	106/111	110/111	116/111	Fractionation relative to reagent cadmium (‰ per mass unit)	
No. of data sets = 18	0.33170 (0.00021)	0.15260 (0.00010)	0.08273 (0.00006)	+1 5	

*See Table 1.

†Source of sample: Dr G. Kurat, Vienna Museum for Natural History.

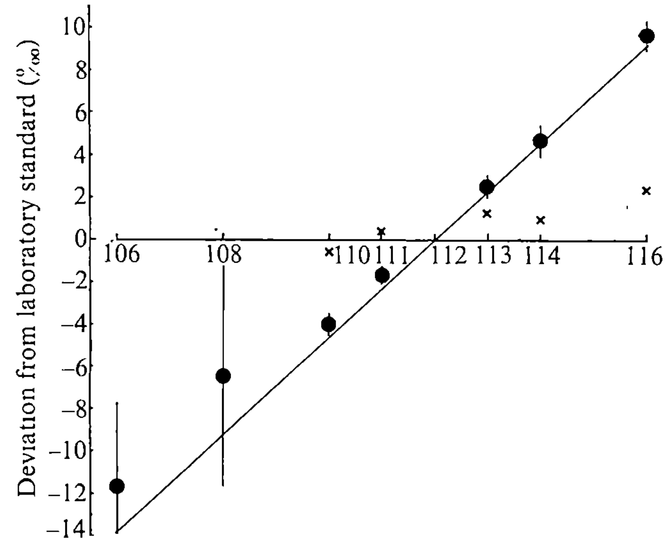
‡The observed enrichment beyond that expected for linear isotope fractionation is probably due to ^{116}Sn contamination Such interferences may be resolved when a double spike is added by selecting 114 instead of 116 for computations

in this meteorite. This may be due to dilution of a fractionated Cd component in the meteorite with an unfractionated component. It is also interesting to note that the Cd content of Tieschitz (values of 75–203 ng g^{-1} have been obtained so far) is substantially lower than that in Brownfield (408 ng g^{-1}) but unusually high when compared with other H chondrites[3]. The chemical inhomogeneity is not unexpected owing to the primitive nature of the H3 chondrites. (Further analyses suggest that the concentration of 408 ng g^{-1} for the bulk meteorite is more representative of Brownfield than that previously reported by us[3].)

Linear isotope fractionation has only been detected in two of the 22 meteorites from various classes examined in this study.

At present we can only speculate on the origin of the anomalous Cd. Arrhenius and Alfvén[5] have indicated several mechanisms which could lead to isotope fractionation during the formation of the Solar System and one of these may account for the observed anomaly. For instance, in chemical species with high re-emission coefficients, such as Hg and the noble gases, efficient isotope separation can be achieved by a multiple desorption process involving impingement, diffusion and desorption from the solid surfaces of a growing condensate. Arrhenius and Alfvén[5] imply that the Hg anomaly observed by Reed and Jovanovic[6] could be attributed to this mechanism. Although Brownfield was not measured by Reed and Jovanovic, it is interesting to note that Tieschitz yielded a 510% enhancement in ^{202}Hg/^{196}Hg ratio. Since Cd has a high ionisation potential and is also volatile, this mechanism may also lead to isotope fractionation of Cd.

Fig. 1 Isotope data for the Brownfield chondrite. ●, Mean of the unspiked analyses shown in Table 1. The error limits indicate two standard errors for the mean. ×, Result of diluting a sample of Brownfield with laboratory reagent Cd. The line represents a fractionation of 2.3‰ per mass unit which is obtained by independent measurements using a double-spiking technique.



Lewis *et al.*[7] have reported the presence of fractionated noble gases in chromite from Allende. Ne, Ar and Kr were enriched in the heavy isotopes whereas Xe was enriched in the lighter isotopes. They suggest that fractionation may have occurred during trapping of the gases from the solar nebula. An examination of Cd in chromite may therefore be profitable.

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Evidence for two discrete centres in Skye

ONE of the best-developed igneous areas in North-West Britain is on the Isle of Skye: in addition to extensive flood basalt lavas and their feeding dykes there are three adjacent intrusive centres across the south-east of the island[1]. The Eastern and Western Red Hills, usually identified as two almost contemporaneous centres, comprise surface granite outcrops in a mass 10 km across, intruded against the older basic-to-ultrabasic Cuillin centre which is of similar size and lies to the west (Fig. 1*a*). Using aeromagnetic, gravity and isotopic evidence we will argue that there are two structurally discrete centres at depth, which probably were intruded several million years apart.

Bott and Tuson[2] have reported a large gravity anomaly over the Skye centres of ∼50 mgal above the regional value (Fig. 1*b*). They interpreted this as due to a conical mass of gabbro widening down to a depth of 15 km, underlying all three centres and outcropping as the Cuillin, with the granitic Red Hills being no more than a veneer, perhaps 2 km thick. The aeromagnetic map[3] shows, however, a negative anomaly of ∼600 nT over the Cuillin, and a roughly equal but positive one over the Red Hills. Though the former can be accounted for if the Cuillin have a reversed remanent magnetisation (common in the province[4,8,18]) the latter cannot be identified with the Red Hills because the anomaly is no less prominent on the filtered aeromagnetic map[5] (Fig. 1*c*) which only registers bodies deeper than ∼5 km in the Skye area[6]. We therefore propose that there are two basic masses underlying the centres of Skye, having opposite magnetic polarities but sufficiently close together to appear as a single gravity anomaly.

It is inherent in our postulate (twin deep-seated masses which cannot be resolved by gravity) that a detailed interpretation is not possible with the present data. Indeed, simple calculations assuming two deep vertical gabbro 'cylinders' of 16-km radius in contact, as suggested in cross section by the magnetic map (Fig. 1c), have shown that only a single gravity anomaly would be resolved. To satisfy the observed magnetic anomalies we must assume that the induced magnetisation in these cylinders is small compared to their remanence[7]. If the latter has an inclination of 60°N, appropriate for an early Tertiary age[8], a strong positive anomaly would be observed to the south and a weak negative to the north at the Red Hills, conversely for the reversely magnetised Cuillin root. Only the strong anomalies can be identified clearly in Skye, but in spite of this it is clear that the magnetic anomalies can be satisfied only if two opposite magnetised bodies are present.

Comparable strong positive and negative magnetic anomalies are not observed on the filtered maps for the other Tertiary centres, and the question arises in what way Skye differs. One possibility is that Skye contains centres formed at two distinct times. Field evidence shows that the succession commenced with the flood basalts; then followed the Cuillin centre which coincided with the later lavas, and in turn was succeeded by the bulk of the (basic) dyke swarm; last came the Western, then the Eastern, Red Hills, but there is no field evidence to determine the intervals in the succession, which have been assumed to be short[9]. Rb–Sr isochron dates on the Red Hills are 52±3 Myr (ref. 10) and 51±4 Myr (ref. 11), and all the 24 K–Ar dates of Kelly[12] are <55 Myr, except for two (59.2±0.7, 59.3±1.0 Myr). There is no evidence for a detectable difference in age between the Eastern and Western Red Hills. The Cuillin are undated, and the only other relevant dated bodies are the lavas, for which the data are equivocal Kelly[12] found that most of his lava samples also gave K–Ar ages <55 Myr. It is, however, believed from oxygen isotope work[13] that the southern part of Skye was subjected to hydrothermal alteration caused by the heat of the centre or centres, and it may be significant that the one sample which came from a locality where the oxygen isotope ratio was normal, gave an apparent age of 59 Myr. Unpublished data from this laboratory on lava samples from the north of Skye also include some samples of higher ages. Thus a possible interpretation of the radiometric evidence is that the lavas—and therefore the Cuillin centre—were intruded ~59 Myr ago, but were extensively 'overprinted' by the heat and chemical changes associated with the Red Hills centres which were not emplaced until ~53 Myr ago. Such conditions probably would not have remagnetised the adjacent rock[14] and, anyway, the magnetism in question here is deep seated.

This date for the lavas gains support from indirect evidence· the dispositions of the dyke-swarms of Skye, Mull and Arran strongly suggest that they were contemporaneous[15,16]. The dykes of Mull mostly pre-date the Loch Bà centre (60.4±1.9 Myr, Rb–Sr isochron[17]), whereas those of Arran came between the closely contemporaneous centres (Northern Granite 58.8±0.6 Myr : Central Complex 58.3±2.2 Myr, dated by the ^{40}Ar/^{39}Ar method[18]) and Holy Island (54.9 Myr, Beckinsale, unpublished): all these dates are consistent with the Skye dykes—and hence the Cuillin which preceded them—being ~59 Myr old.

It is a basic assumption of this paper that the Cuillin 'cylinder' acquired its remanence during a period of reversed polarity; an age of 59 Myr is consistent with this, for the lavas of Mull are of similar age and are reversed[19,20].

Strontium and lead isotopic evidence[11,21] suggest that the Red Hills granites were mainly derived by partial melting of Lewisian rocks although the possible effects of hydrothermal alteration[13] throws the isotopic evidence into doubt. Whether these granites were derived by partial melting, or by fractional crystallisation of the underlying basic rock mass, a large heat source is implied. This could not be the mass underlying the Cuillin if it were intruded several million years before; therefore, a second source is needed which we have identified from magnetic anomalies to be a second deep-seated basic mass which cooled during a normal polarity period.

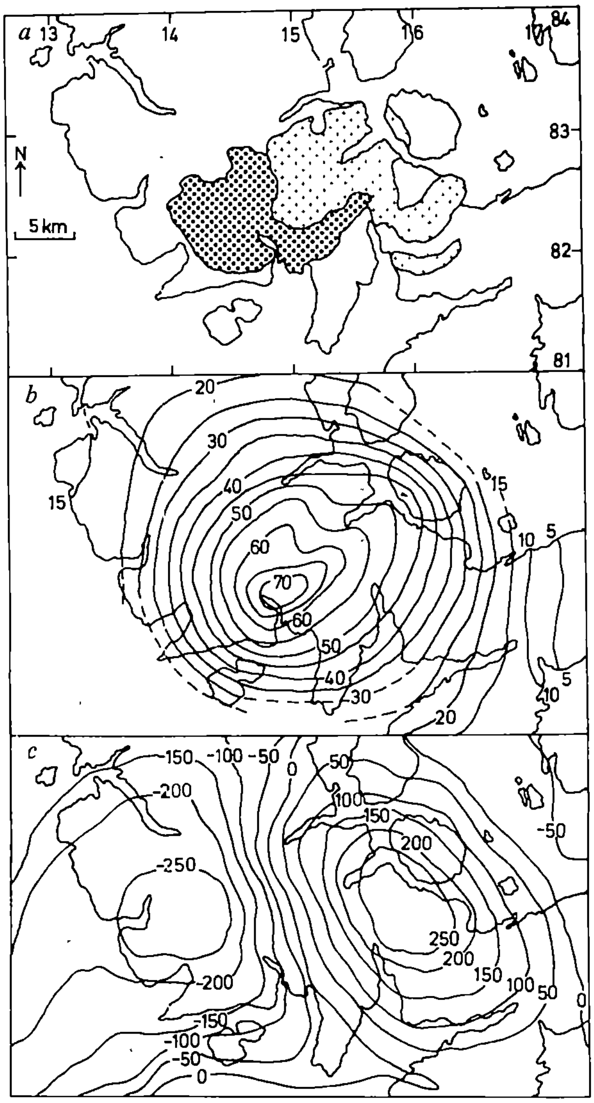

Fig. 1 *a*, Map of southern Skye showing the juxtaposition of intrusive centres at outcrop level[1]. Distances are given in tens of kilometres north and east of the national grid origin. Dotted pattern, Cuillin gabbro; crossed pattern, Red Hills granite; blank areas, Tertiary lava and older rocks. *b*, Gravity anomaly[2] on the same scale with contours at 5-mgal intervals. *c*, Total field aeromagnetic anomalies filtered at 3.33-km intervals[5], representing anomalous material buried beneath ~5 km (ref. 6). Contours at 50-nT intervals.

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Promotion and retardation of heat transfer by electric fields

I REPORT here on the effects of an electric field on heat transfer. The effects depend on the applied voltage, the shapes, number and locations of the electrodes, and the frequency of the electric field[1,2].

The vaporisation of water is enhanced remarkably in an electric field: at 12 °C, the rate increases by up to 40–50 times[2]. This phenomenon suggests that heat transfer may well be influenced by electric fields; the experiments reported here show this to be the case. The rate of heat transfer in a gas can be increased by up to 1.5 times, in liquids by up to 2.0 times, and in solids by up to 1.6 times, according to the positioning of the electrodes and the strengths of the electric fields.

Under the influence of electric fields another interesting phenomenon has also been observed: the rate of cooling can be retarded. If an electrode is located within material that is to be heated, cooling proceeds more slowly after the heat source is removed. Cooling takes place 1.5 to 2.0 times more slowly than in the absence of an electric field.

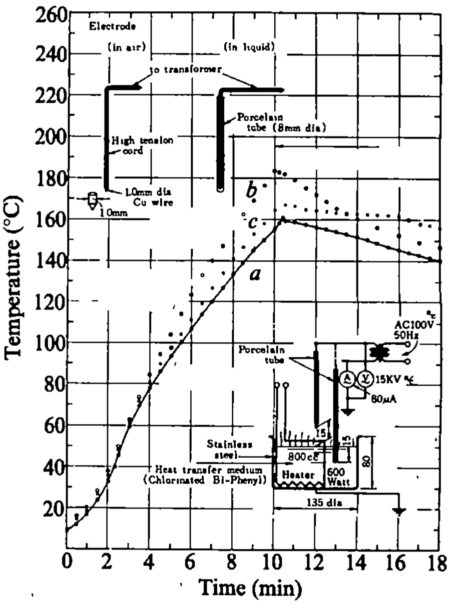

Fig. 2 Heating and cooling curves of chlorinated biphenyl (liquid) contained in a stainless steel beaker (diameter 135 mm), with the heating source placed within the liquid: *a*, in the absence of an electric field; *b*, in the presence of an alternating field (15 kV; 80 µA) with the electrode (Cu wire, diameter 1.0 mm) outside the liquid; *c*, in the presence of an alternating field (15 kV; 80 µA) with the electrode (Cu wire, diameter 1.0 mm) inside a porcelain tube within the liquid. After heating for 10 min, temperatures of 161, 184, and 167 °C were attained in cases *a*, *b*, and *c*, respectively. When the heat source was removed (after 10 min) the temperatures fell in 8 min to 140, 146, and 156 °C, respectively, in cases *a*, *b*, and *c*. In cases *b* and *c* the alternating current remained on, and cooling was 1.7 times slower (*b*) and 1.8 times slower (*c*) than in case *a*.

The restriction of heat transfer can be compared to the behaviour of current flow in an electrostatic transistor.

The methods and apparatus used in the experiments are described in Figs 1–3. It should be noted that no energy is absorbed from the electric field, except for 1.5–2.0 W as a result of ionisation or leakage.

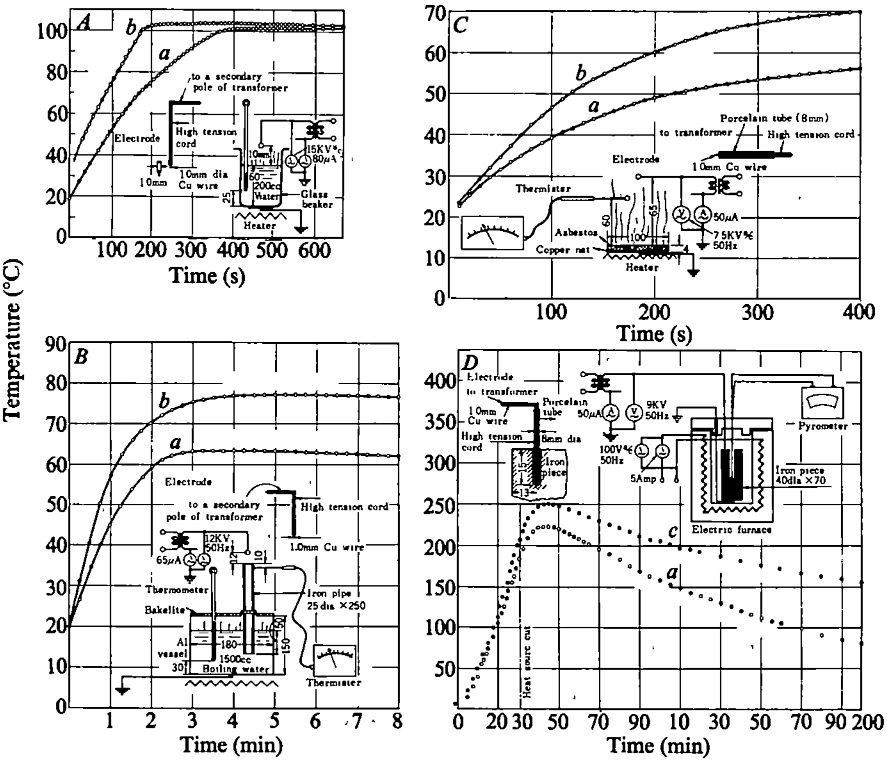

Fig. 1 *A*, Heating curves of 200 cm³ water contained in a glass beaker: *a*, in the absence of an electric field; *b*, in the presence of an alternating electric field (15 kV; 80 µA) with the electrode (Cu wire, diameter 1.0 mm) placed 10 mm above the water. Boiling point is attained twice as quickly in case *b*. *B*, Heating curve of an iron pipe (diameter 25 mm, length 250 mm): *a*, in the absence of an electric field; *b*, in the presence of an alternating field (12 kV; 65 µA) with the electrode (Cu wire, diameter 1.0 mm) placed 12 mm above the end of the pipe. The pipe was heated by placing its lower end in 1,500 cm³ of boiling water contained in an aluminium vessel insulated with a bakelite lid. In the presence of the electric field a temperature of 76 °C was obtained (*b*), compared with a maximum temperature of 63 °C with no electric field (*a*). *C*, Heating curves of free air: *a*, in the absence of an electric field; *b*, in the presence of an alternating field (7.5 kV; 50 µA) with the electrode (Cu wire, diameter 1.00 mm) placed 65 mm above a heated asbestos mat. Temperature of the air between the electrode and the mat reached 70 °C in case *b*, as opposed to 56 °C in case *a*. *D*, Heating and cooling curves of an iron cylinder (diameter 40 mm; height 70 mm): *a*, in the absence of an electric field; *b*, in the presence of an alternating field (9 kV; 50 µA) with the electrode (Cu wire, diameter 1.0 mm) inserted 15 mm into the cylinder. The cylinder was heated within an electric furnace, and in case *b* cooling was 1.6 times as slow as in case *a*.

If the nature of the promotion of heat transfer is considered thermodynamically, it can be regarded as a phenomenon in which materials (fluids) change their qualities so as to absorb more heat energy from their surroundings or transfer more heat energy within themselves.

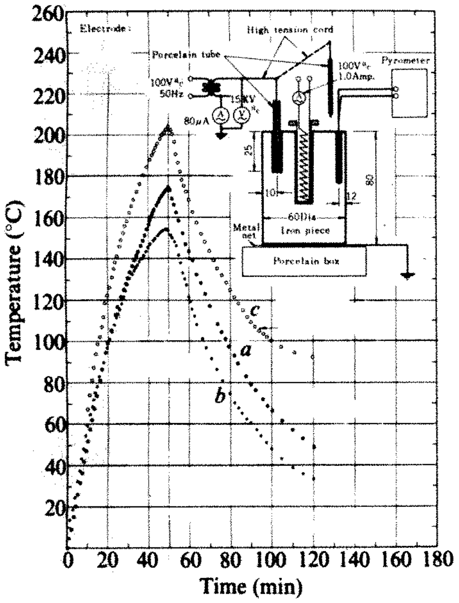

Fig. 3 Heating and cooling curves of an iron cylinder (diameter 60 mm, height 80 mm) with the heat source placed within the cylinder: *a*, in the absence of an electric field; *b*, in the presence of an alternating field (15 kV, 80 μA) with the electrode outside the cylinder; *c*, in the presence of an alternating field (15 kV, 80 μA) with the electrode (Cu wire, diameter 1.0 mm) inserted 25 mm into the cylinder. After heating for 50 min, temperatures of 175, 155, and 205 °C were attained in cases *a*, *b*, and *c*, respectively. In this case, when the heat source is removed cooling is more rapid in case *b* than in case *a;* a similar phenomenon occurs with liquids, however, if only small quantities of heat are supplied initially.

The phenomenon could be investigated further through studies of infrared absorption patterns[4] and increases of vapour pressures[5,6].

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Microfossils from a newly discovered Precambrian stromatolitic iron formation in Western Australia

THE Nabberu Basin is a newly discovered sedimentary basin of probable early Proterozoic age in Western Australia[1-3] (Fig. 1). It covers an area of ~60,000 km², and contains ~6,000 m of shallow-water clastic and chemical sediments. It is essentially unmetamorphosed in the south-east but is metamorphosed to granulite facies in the west, where folding and deformation are more pronounced.

In the middle of the Nabberu Basin sequence is the Frere Formation—~2,000 m of alternating banded iron formation and shale, with thin stromatolitic dolomite at its base. The iron formations are granular and are very similar to those of the Animikie Basin and Labrador Trough in North America[4,5]. Within the Frere Formation at a location of about 2.5 km north-east of Camel Well in the south-eastern part of the basin (latitude 26°08′, longitude 121°18′), a thin oncolitic iron formation is interbedded with peloidal and oolitic ferruginous chert. The oncolites and associated stratiform stromatolites contain the abundant microfossils[6,7]. Microfossils, as yet unstudied, are also present in the Windidda Formation, overlying the Frere Formation, in thin, black to grey apatitic and sulphidic cherts near Tooloo Bluff (latitude 26°27.5′, longitude 122°14′).

The iron formation microfossils are of special interest because the assemblage is almost identical to that of the Gunflint Iron Formation of Ontario[8], but is different in many respects from other described Precambrian assemblages[9]. This similarity may result from the facies similarity or from the probable penecontemporaneity of the two deposits, which we shall discuss. This is the first time that two particularly distinctive and comparable assemblages of Precambrian microfossils have been found widely separated on two continents and the biostratigraphic significance of this discovery must be assessed. The nature of the occurrence of microfossils within oncolites indicates a benthonic mode of life, conflicting with an interpretation[10] of a planktonic mode for several of the same taxa from the Gunflint. Here we briefly describe the Nabberu Basin microfossils and consider the questions and comparisons outlined above.

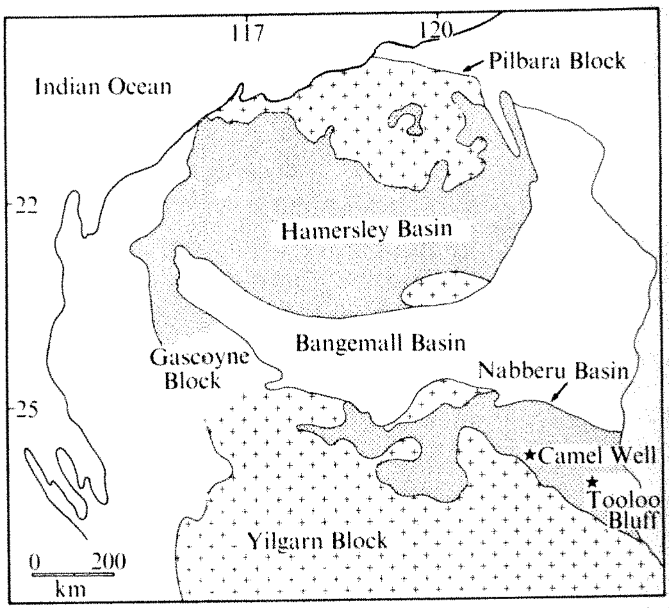

Fig. 1 Locality map. Crosses represent Archaean rocks, stippled areas are early Proterozoic sedimentary basins; the Bangemall Basin is Middle Proterozoic; the remaining unornamented areas are underlain by Phanerozoic rocks.

The Nabberu sediments unconformably overlie the Archaean granite/greenstone basement of the Yilgarn Block, and are unconformably overlain by sediments of the approximately 1,100-Myr-old Bangemall Basin (Fig. 1). Glauconites from the basal Yelma Sandstone of the Nabberu Basin give an average minimum age of 1,700 Myr (ref. 3), which is about the time of tectonism of the nearby Hamersley Basin. There is evidence to suggest that the Nabberu Basin can be traced through the high grade Gascoyne Block at its western extremity into the sediments of the Hamersley Basin, which are well dated as approximately 2,000 Myr old[11]. Thus the Frere Formation of the Nabberu Basin seems to be approximately the same age as the Gunflint Iron Formation.

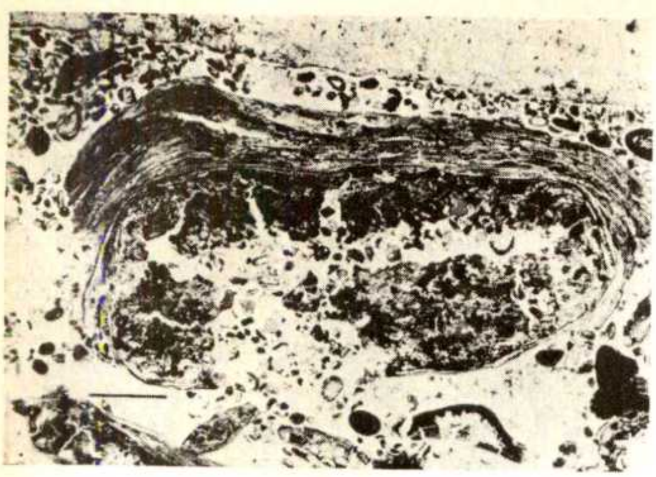

Fig. 2 Microfossiliferous oncolites and granules, Frere Formation. Scale bar represents 2 mm. Thin section BHP 5319D3. All specimens are kept in the Bureau of Mineral Resources, Geology and Geophysics, Canberra.

The microfossils occur within discoidal and subspherical stromatolites (that is, oncolites), stratiform stromatolites, and large granules (peloids) (Fig. 2). Filaments 1–2 μm wide and up to hundreds of microns long (here identified as *Gunflintia minuta* Barghoorn) are abundant and ubiquitous in the stromatolites (Fig. 3, *a* and *b*), and most are contorted and randomly oriented. Not uncommonly, however, groups of filaments are aligned roughly parallel to the stromatolite laminae, and occasionally such groups can be followed into contiguous laminae where they turn to become approximately perpendicular to the laminae. The organisms represented by these filaments seem to have constructed the stromatolites. The filaments, which are preserved as segmented rods of iron oxide, sometimes occur within tubes of iron oxide (Fig. 3*c*). These and other non-septate tubes 4–9 μm wide (Fig. 3*d*) without internal filaments (trichomes) are interpreted as the former mucus sheaths of cyanophytes, as is *Animikiea septata* Barghoorn from the Gunflint[10]; the Frere Formation tubes have a slightly different range of sizes and are less clearly annulate than the Gunflint forms and are therefore referred to a new species of *Animikiea*.

Rosettes of filaments occur singly and in clusters within the stromatolites and granules (Fig. 3*e–g*). Each rosette consists of 5–20 filaments of variable width (0.5–1.5 μm); the width of the rosettes is 5–25 μm. The filaments are unbranched (here referred to *Eoastrion simplex* Barghoorn) or, rarely, dichotomously branched (referred to *E. bifurcatum* Barghoorn). The degree of mineralisation of the filaments is variable; they are often heavily encrusted with iron oxides and so coalesce into a subspherical central body. They closely resemble the extant benthonic iron- and manganese-precipitating bacterium *Metallogenium personatum*[12], as has been noted previously[13].

Hollow spheres and ellipsoids of iron oxide referable to the genus *Huroniospora* Barghoorn occur singly or, more commonly, in groups with separate individuals (Fig. 3*h–k*). They range in width from 2.5 to 12.0 μm, with a mode and a mean of 6 μm. There are several examples of apparently dividing cells (Fig. 3*i* and *j*). The three species originally described from the Gunflint were distinguished by their wall textures and thicknesses, features that are now considered as possibly secondary[10]. The species have not yet been redefined. It is notable, however, that many of the cells in the Frere Formation resemble those in the Gunflint originally called *H. psilata* Barghoorn.

One of the most distinctive microfossils in the Gunflint is *Kakabekia umbellata* Barghoorn. This is also present, but relatively rare, in the Frere Formation (Fig. 3*l–o*), where it occurs separately or in small groups within the stromatolites. An extant homoeomorph has been described from soils[14]. One example has been found of a structure that resembles the branched tubular fossil *Archaeorestis schreiberensis* Barghoorn from the Gunflint, but identification must wait until more samples have been discovered.

In the Gunflint Iron Formation there are two different microfossiliferous chert facies: "One, a thin bedded chert, contains what is interpreted as a planktonic assemblage of microorganisms dominated by *Kakabekia umbellata* Barghoorn, *Eoastrion simplex* Barghoorn, and *E. bifurcatum* Barghoorn"[10]. The other is stromatolitic, and thus benthonic, and is dominated by *Gunflintia minuta* and *Huroniospora* spp., with rarer specimens of *G. grandis* Barghoorn, *Animikiea septata* and other forms. The Frere Formation stromatolitic assemblage described here contains

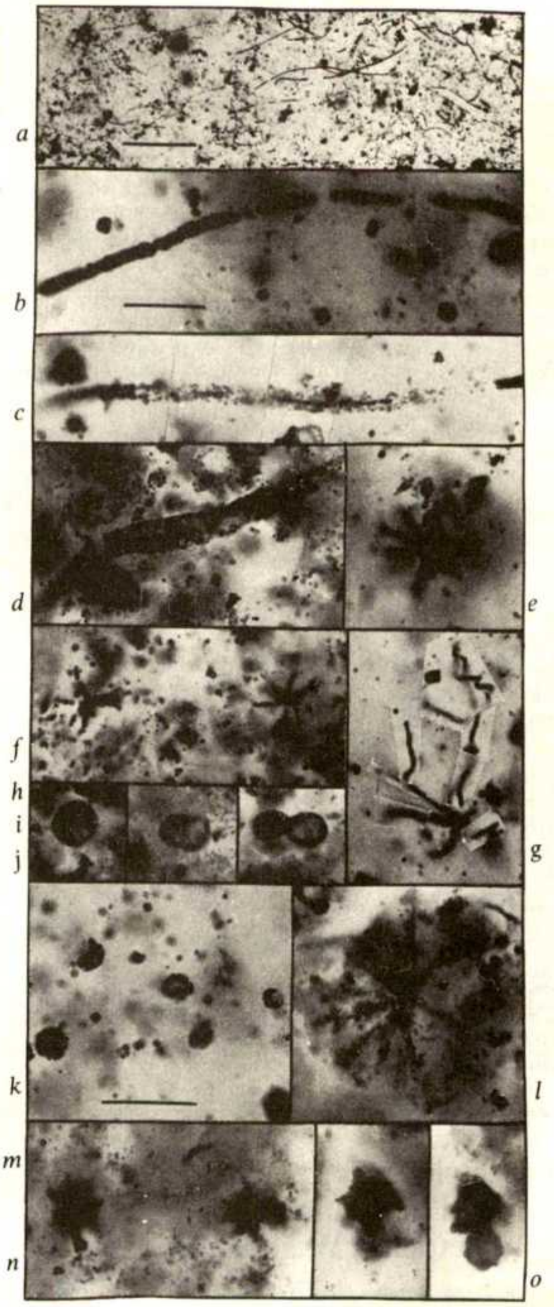

Fig. 3 Microfossils from the Frere Formation. All photographs are of petrographic thin sections of ferruginous chert in transmitted light. Scale bar in *a*, 100 μm; in *b*, 10 μm; in *k*, 20 μm (that in *k* applies to photographs *c–o*). *a* and *b*, *Gunflintia minuta*; *c* and *d*, *Animikiea* sp. nov., in *c* with an internal *G. minuta* trichome; *e*, *f* and *g*, *Eoastrion simplex*; *h–k*, *Huroniospora* spp.; *l–o*, *Kakabekia umbellata*, photographs *n* and *o* are of the same fossil at 2 different focal levels. All are in thin section BHP 5319D1.

representatives of both these Gunflint assemblages: it resembles the Gunflint stromatolite assemblage in that it is also dominated by *G. minuta* and *Huroniospora* spp., but in addition contains abundant *E. simplex* and rare *E. bifurcatum* and *K. umbellata*, considered by Awramik[10] to have been planktonic (although the basis for this interpretation has not yet been published). The occurrence of *E. simplex* in clusters within the Frere Formation oncolites strongly suggests that at least in the Nabberu Basin this organism was benthonic, an interpretation that is consistent with the fact that the most similar extant organism, *Metallogenium personatum*, inhabits lacustrine muds[12]. Furthermore, at least some extant rosette-forming bacteria attach to a substrate before forming rosettes[15]. Similarly, *K. umbellata* may also have been benthonic since it occurs in clusters and the only known extant homoeomorph inhabits soils. The search for microfossils in non-stromatolitic laminated cherts of the Frere Formation has been so far unrewarded, and only dubious microfossils have been reported[16] from the possibly correlative non-stromatolitic, non-granular iron formations of the nearby Hamersley Basin.

The stromatolites of the Gunflint Iron Formation and the Frere Formation seem likely to have formed in very similar environments. Both are composed of almost certainly primary silica and iron compounds and occur with granules and ooids which suggest a turbulent sub-aqueous environment. These palaeoenvironmental similarities may be sufficient to explain the near identity of the two assemblages of microfossils and their differences from older and younger assemblages[7,17], since these older and younger assemblages are almost entirely from fine grained siliciclastic rocks and secondary cherts in stromatolitic carbonates. Such a close similarity of two probably penecontemporaneous microfossil assemblages, however, certainly encourages further investigation of the biostratigraphic usefulness of Precambrian microfossils. It is not possible to consider this aspect in detail here, but it is evident that the discovery of the Frere Formation microbiota will prove important in studies which use chert microbiotas in Precambrian biostratigraphy.

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Population genetic, social, linguistic and topographical relationships in north-eastern Arnhem Land, Australia

GENETIC differentiation has been found among Australian Aboriginal tribes[1], but no previous attempt has been made to assess intratribal differentiation. We report here on genetic differentiation within an Aboriginal 'tribe' related to linguistic, social and topographical components of the population, together with confirmation of the importance of drainage systems as natural barriers to gene flow. An estimate of inbreeding in a traditionally structured Aboriginal local group is given.

In an earlier intertribal study[1] we found that genetic differentiation was highly correlated with linguistic and sociocultural divisions. More recently we have concentrated on genetic differentiation at the level of the territorial and marriage unit within the Yolngu (a collective term for the dialects of the region (B. Schebeck, personal communication)) of the north-eastern Arnhem Land region of the Northern Territory of Australia (Fig. 1). This region is of considerable anthropological importance[2–4]. It is one of few remaining areas of Aboriginal Australia where the simultaneous effect of ecological, demographic, sociocultural, linguistic and biological influences on phenotypic variation can be investigated. The Yolngu population is consistent with a biological definition of tribe[5] being at least 90% endogamous. They are organised into localised totemic clans and dialect units which are territorially based and exogamous[4]. They now number about 2,400 and occupy a territory of about 26,000 km^2, bounded by coastline to the north and east and river systems to the west and south (Fig. 2).

This report concerns two dermatoglyphic traits, fingerprint pattern index and total ridge-count, both strongly heritable[6]. In addition, phenylthiocarbamide (PTC) taster ability, red–green colour blindness, handedness, earlobe form, hairy pinnae and skin colour were studied and generally supported our findings from the dermatoglyphic

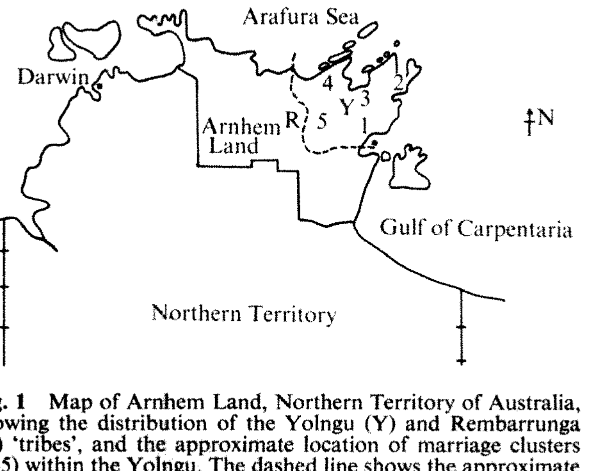


Fig. 1 Map of Arnhem Land, Northern Territory of Australia, showing the distribution of the Yolngu (Y) and Rembarrunga (R) 'tribes', and the approximate location of marriage clusters (1–5) within the Yolngu. The dashed line shows the approximate boundary of the Yolngu.

Table 1 The relationship of the various population units to the genetic structure of the Yolngu, as assessed by dermatoglyphics

Unit of comparison	No. used in analysis	No. of comparisons	No. significantly different at probability level*				Percentage of comparisons significant
			0.05	0.02	0.01	0.001	at ⩽ 0.05
Dialect groups	7	21	—	2	1	2	24
Ceremonial/'war' groups	6	15	3	1	—	—	27
Marriage clusters	5	10	3	2	1	—	60
Drainage divisions	6	15	—	4	1	7	80

*Pairwise *t* comparisons.

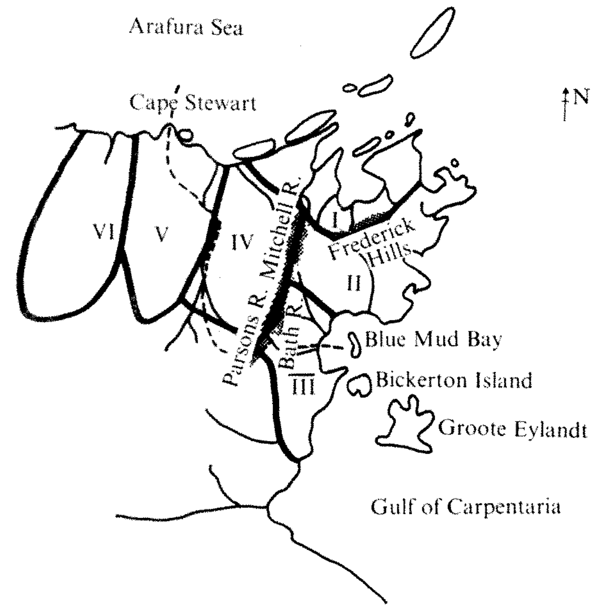
data. A total of 744 Yolngu were sampled, that is, 31% of the population. In addition to biological information, data were obtained on the social affiliation of the parents and spouse(s) of each subject, their ages and places of birth, together with information on marriage networks, genealogies and the distribution of the territorial units.

Table 1 indicates some genetic differentiation among the dialect groups of the Yolngu. Nine such groups, of which we have adequate data for seven, each consisting of a number of dialect units, are recognised by both linguist and Aboriginal, all but two being potentially endogamous[7]. Each statistically significant comparison involved the same dialect group, which suggests that the dialect groups form an intermarrying complex with no genetically significant endogamy within them, although there is some support for the suggestion of two linguistic "divisions" among the dialect units of the Yolngu[8].

Total ridge-count was analysed according to dialect unit affiliation within recognised ceremonial/fighting groups. These groups were said by Aboriginal informants to be "tied together for maridjama (fighting) and manikaj (ceremonial singing)". The analysis revealed statistically significant differences, all of which were accounted for by the 'fighting' group known as the Balamumu, a collection of dialect units in the Blue Mud Bay area (Fig. 2). This suggests that the Balamumu, at least, functions largely as an endogamous unit, being genetically distinguishable.

Marriage 'clusters' were recognisable from the marriage data and correspond to those dialect units among which there was at least 70% endogamy. The dialect units that intermarried most generally had adjoining territories.

Fig. 2 Map of north-eastern Arnhem Land, Northern Territory, showing drainage divisions (I–VI) and some topographical features. Solid lines indicate major river systems, and the dashed line is the approximate boundary of the Yolngu.



There were similarities between the structure of the ceremonial/fighting groups and marriage 'clusters', suggesting that the former groups are based on marriage relationships. The marriage cluster centred on the Blue Mud Bay region (Fig. 1, cluster 1) was distinctive, an observation consistent with our findings for the Balamumu fighting group.

The marriage clusters are also associated with drainage divisions. Peterson[9] highlighted the role of drainage basins in the cultural and social differentiation in Aboriginal Australia. He noted that watersheds might have acted as natural barriers to communication. Independently we considered the drainage basins as units for analysis: our results (Table 1) indicate that these natural divisions are important in terms of genetic differentiation. In this analysis, data are included from some other neighbouring tribes, as drainage basins overlap the Yolngu territory (Fig. 2).

For the dermatoglyphic traits the greatest and most statistically significant difference was between the Aborigines living in the Blue Mud Bay area, and those living at the western boundary of the Yolngu. These two groups are separated topographically by a complex of low ranges (Fig. 2) which almost certainly restrict intermarriage although important ceremonial links exist. There is also evidence for gene flow from some neighbouring tribes into the western Yolngu group. This no doubt increased the extent of the differences between the groups. We found for the fingerprint, and other biological traits, that the three Yolngu dialect units along the western boundary are not significantly different from their immediate western tribal neighbours, the Rembarrunga (Fig. 1). There is also no sharp linguistic boundary to the west (B. Schebeck, unpublished).

We concentrated on a traditionally structured local group in the western Yolngu region made up of about 60 people belonging mainly to two intermarrying dialect units. The average level of inbreeding for this local group was estimated to be 0.02 from genealogical data—a value similar to that for Central and South American Indian populations[10]. The highest individual coefficient of inbreeding was 0.07 from data over five generations.

In conclusion, the Yolngu function as a marriage network of endogamous units within a broad cultural and linguistic complex. At both the 'tribal' and regional level these endogamous units seem to be closely related to the drainage basin. This finding supports Warner's[2] description of "Murngin" (Yolngu) society as a "confederacy" of several "tribes", at least when the term 'tribe' is used in the biological sense.

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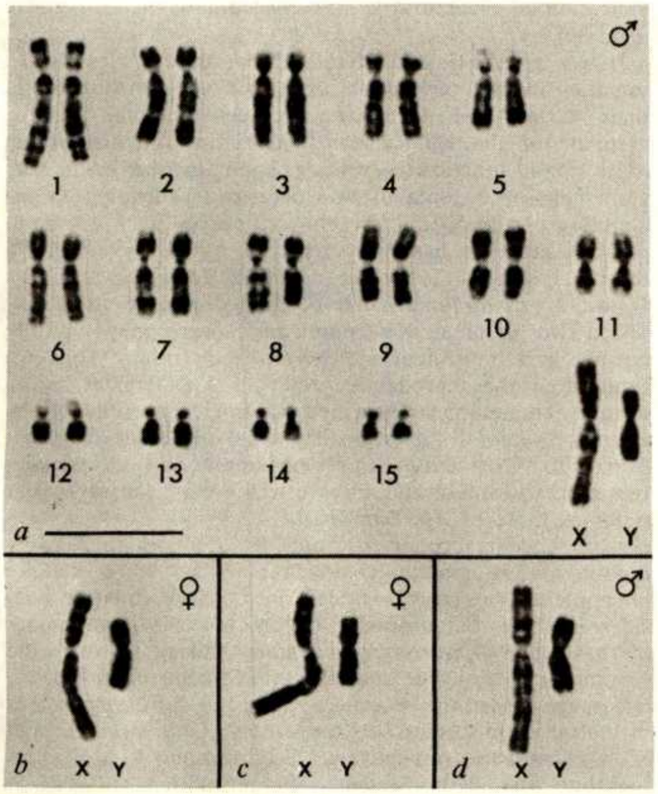
Fertile XX- and XY-type females in the wood lemming *Myopus schisticolor*

THE wood lemming (*Myopus schisticolor* Lilljeborg), a small rodent inhabiting mossy forests of northern coniferous areas of Eurasia, is unique with respect to sex ratio, showing a definite prevalence of females, and with respect to the occurrence of two types of female, one producing daughters only, the other producing progeny of both sexes[1,2]. We thought that these phenomena might be associated with chromosome constitution, and as the chromosomes of this species were poorly known, we have made a detailed study of its karyotype. Some additional observations, pertinent both to the occurrence of chromosome variation and to reproduction, will be reported elsewhere. We report here two remarkable findings: (1) the existence of two types of fertile female with different somatic sex chromosome constitutions—the orthodox female complement, XX, and the male complement, XY, and (2) evidence for selective non-disjunction in XY females, leading to the formation of X-type egg cells only.

The material for the chromosome study consisted of 29 specimens collected in four areas of Sweden, and some 180 descendants of animals trapped in a fifth locality in Sweden (Table 1). Mitotic chromosomes were studied in direct preparations from bone marrow, and in fibroblast cultures from lung, heart or embryonic tissues. Cells were usually processed by the conventional air drying procedure and the preparations were stained with orcein, Giemsa or the fluorochromes 33258 Hoechst and acridine orange. G and C staining[3,4] were applied to preparations from most of the animals. The late replication pattern of the chromosomes was examined by autoradiography. Meiosis was studied in testes from eight males and in ovaries from 19 females. In males, meiotic chromosomes were studied by the method of Evans *et al.*[5]. For females the method described by Tsuchida and Uchida[6] was used. Primarily diakinesis and metaphase I were evaluated. Histological sections of the female reproductive tract were prepared in the usual manner from most animals studied.

Fig. 1 G banded chromosomes of *M. schisticolor*. *a*, Karyotype of a male. *b* and *c*, XY sex chromosomes of a female. *d*, XY sex chromosomes of a male. Scale = 10 μm.



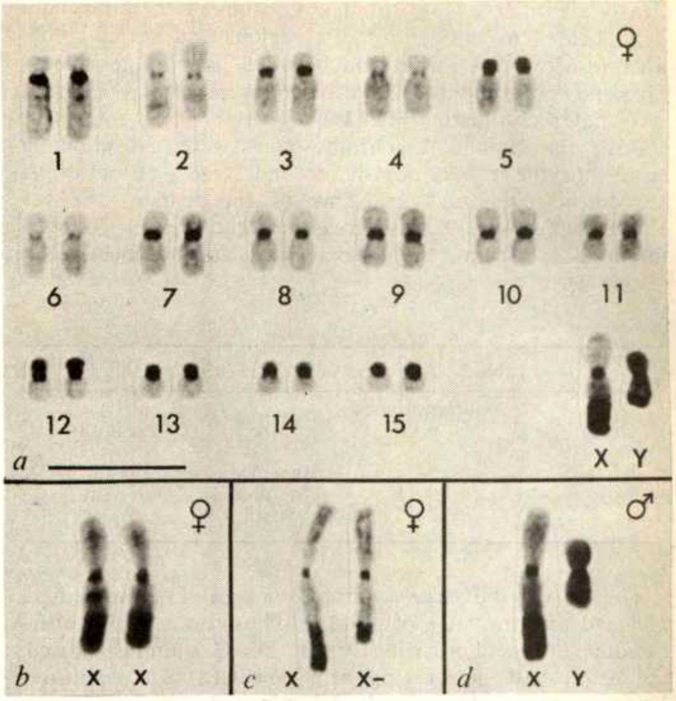


Fig. 2 C-stained chromosomes of *M. schisticolor*. *a*, Karyotype of an XY female. Note the absence of centromeric heterochromatin in nos. 2, 4 and 6. Pair no. 2 is dimorphic in this individual. *b*, XX sex chromosomes of a female. *c*, Sex chromosomes from a female with one normal and one deleted X. *d*, XY sex chromosomes from a male. Note the same staining pattern as in the XY female. Scale = 10 μm.

The chromosome number of the animals was $2n=32$ (Fig. 1), confirming Matthey's study[7]. The autosomes were divided into three groups on the basis of size and arm ratio (nomenclature according to Levan *et al.*[8], Table 2). Chromosomes 5 and 12 had satellites attached to their short arms. After G staining, every autosome pair was

Table 1 Data on wood lemmings (*Myopus schisticolor*) from Sweden

Trapping locality and year		Animals studied		Sex chromosomes	
		Sex	No.	XY	XX
Våmhus, Dalarna	1973	Male	—		
(61°15′N; 14°15′E)		Female	3*		3
Tandsjöborg, Dalarna	1973	Male	1	1	
(61°45′N; 14°30′E)		Female	—		
Årjäng, Värmland	1973	Male	3	3	
(59°15′N; 12°15′E)		Female	4	4	
Hällnäs, Västerbotten	1974	Male	5	5	
(64°15′N; 19°45′E)		Female	13	4	9
Nås, Dalarna	1963†	Male	17	17	
(60°30′N; 14°30′E)		Female	161	74	87

*Pregnant female with four embryos. Chromosome preparations were obtained from the mother and two of the embryos.

†The animals studied are from a colony kept by F.F. since 1963. They are descendants of five males, and ten females trapped at Nås.

clearly identifiable (Fig. 1*a*). The C bands of the autosomes were located in the centromeric regions and varied somewhat in size both within and between populations. Three pairs, however, numbers 2, 4 and 6, were regularly devoid of C heterochromatin (Fig. 2*a*).

Both the X and Y chromosomes were exceptionally large, but the Y was not the second largest chromosome as Matthey[7] reported. In some of the females the sex chromosomes were XX, whereas in others they were indistinguishable from the male sex chromosomes, XY (Table 1).

Table 2 Autosomes of *M. schisticolor*

Group	Chromosomes no.	Size	Centromeric position
1	1– 5	Large–medium	sm—st
2	6–11	Medium	m—sm
3	12–15	Small	sm—st

The X was the largest chromosome of the complement and had an arm ratio of 1.5–1.7. Thus it was an m chromosome on the borderline of sm. As C staining showed, the distal half of its long arm consisted of constitutive heterochromatin (Fig. 2*a–d*). It stained darkly by C staining, fluoresced comparatively weakly after staining with Hoechst 33258 and showed late DNA synthesis. Occasionally, a finer substructure of heavily stained bands was seen within the C segment. Sometimes on an X there were faint indications of gaps between the euchromatic and heterochromatic parts and within this latter segment. This observation was possibly related to occasional variations in the size of the heterochromatin: in one XY female it was about two-thirds, and in one X chromosome of one XX female it was only one-third of the normal size (Fig. 2*c*). The centromeric region of the X was C-band positive.

The Y was an m chromosome of medium size, similar to numbers 9–10 but with a somewhat higher arm ratio. These and the following features apply equally to the Y in males and to the Y-like sex chromosome in XY females. The entire Y was dark after C staining, clearly darker than the euchromatic parts of the X, although often less dark than the heterochromatic parts of the X. There were different intensities of staining within the Y, the distal part of the long arm being darkest. The centromeric region of the Y was not particularly heavily stained. The Y synthesised DNA late. Both Y and X showed characteristic G-band patterns, even though the bands in the entire Y, as well as in the distal half of the long arm of X, were less distinct than in the autosomes (Fig. 1).

C and G staining showed that the Y of the females was not a deleted or rearranged X chromosome. This is supported by the following evidence: (1) the 'female Y' always had exactly the same morphology as the 'male Y', including every detail of the banding pattern, and (2) the 'female Y' did not have any of the staining properties of the regular X chromosome.

Further clarification was obtained from an examination of meiosis. In male diakinesis the X and Y chromosomes were associated end-to-end (Fig. 3*a*). The sex bivalent, however, usually had a coiled configuration, not allowing distinct recognition of the two constituents. The structure of the 15 autosomal bivalents, on the other hand, was mostly clear and distinct. At diakinesis of XX females there were 16 symmetrical bivalents, 15 of them autosomal and one—the largest—formed by the two Xs (Fig. 3*b*). The XX bivalent had one or two chiasmata. In all 29 diakinesis figures from 10 XY females, the composition and type of bivalents was the same. Again the large sex-bivalent was clearly identifiable as a symmetrical figure with lateral pairing and one or two chiasmata and with no indication of structural or heterochromatic heterozygosity (Fig. 3*c*).

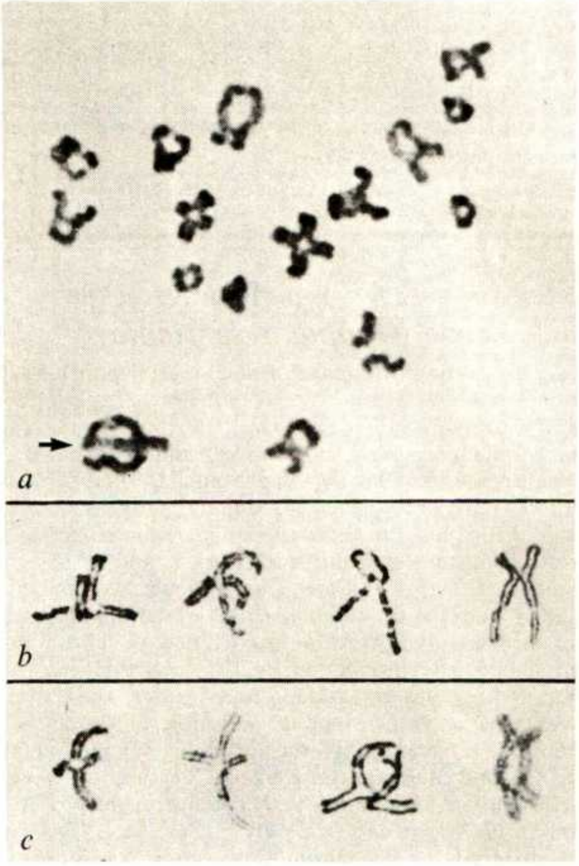


Fig. 3 Male (*a*) and female (*b* and *c*) meiosis in *M. schisticolor*. *a*, Complete cell in diakinesis showing 15 autosomal bivalents and the XY bivalent (arrow indicates the site of end-to-end association between X and Y). *b* and *c*, Sex bivalents from eight cells in diakinesis from XX (*b*) and XY (*c*) females (four and three different individuals, respectively). Note that in both XX and XY females the bivalent is symmetrical and composed of two Xs with one or two chiasmata.

It has to be assumed therefore that the females with a somatic XY set possess an orthodox XX constitution in their oocytes. This discrepancy between the soma and the germline of the XY females necessitates the assumption of a non-disjunctional event, possibly in the primordial germ cells or oogonia of the foetal ovary, leading to the doubling of the X and the elimination of the Y. A comparable situation has been reported by Ohno *et al.*[9] in *Microtus oregoni*, a species with XO females.

Both kinds of female, irrespective of whether they possessed two Xs or an XY complement, were phenotypically normal and indistinguishable from each other. Thus we found that the reproductive tract of XY females had a normal female appearance, and histological sections of the ovaries revealed follicles in different stages of development similar to XX females. Correspondingly, the XY females reproduce normally and their litters are of the same size order as those of XX females.

Is the occurrence of XX and XY females in *Myopus* unique among mammals? As far as we know such a phenomenon has not been described before, but we consider it likely that a similar condition exists in lemmings of the species *Dicrostonyx torquatus* (Pallas), in which the chromosomes of three subspecies have been described[10–13]. We suggest that the so-called x, X_2 and X_3 chromosomes in the three subspecies *D. t. stevensoni*, *D. t. torquatus* and *D. t. chionopaes*, respectively, all correspond to a Y chromosome, just as in *Myopus*. We postulate that both in

Myopus and in *Dicrostonyx* some of the XY animals function as fertile females due to an X-linked gene repressing the male-determining effect of the Y (our work in preparation).

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Epithelial origin of starfish coelomocytes

THE various theories proposed to explain the origin of echinoderm coelomocytes can be classified into two groups, depending on whether they refer to a cytopoietic organ or tissue (Tiedemann bodies[1–3], Polian vesicles[1], axial organ[1,4], connective tissue[5,6] and coelomic epithelium[7–9]) or whether they imply self-replication of free cells[9–11]. So far no theory has been confirmed. We now report that in the starfish (*Asterias rubens*) coelomocytes are produced in the coelomic epithelium.

A. rubens has only one type of coelomocyte[12], which, like the coelomic epithelial cells[13], is easy to label by intracoelomic injection of ferritin (cadmium-free, Calbiochem, final concentration 3–5 mg ml^{-1} of coelomic fluid). The iron is then made visible by applying Perls' reaction[14] on smears and histological sections.

Cuénot's observations[13] are correct: only the coelomocytes and the coelomic epithelial cells absorb ferritin. Injections have shown that there is no transfer of cells from one coelom to another (Table 1). Therefore, the coelomic epithelium which lines the axial organ is not labelled unless ferritin is injected into the axial sinus. Also labelled coelomocytes are never found in the general body cavity after injection into the hydrocoel or the axocoel, and *vice versa*. Contrary to what happens in other echinoderms, the starfish's coelomocytes leave the coelom only to be eliminated into the external medium.

To determine the site of origin of the coelomocytes, two starfish which had been injected with ferritin (into the general body cavity) were bled several times. After the first bleeding, quite a large number of new coelomocytes

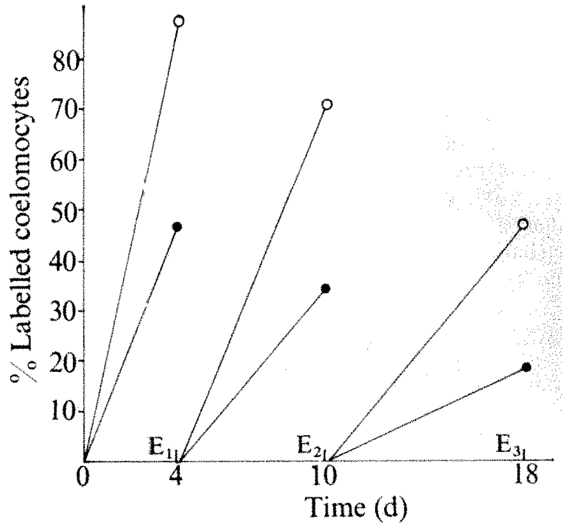

Fig. 1 Production of labelled coelomocytes at each bleeding (E_1, E_2 and E_3) in two starfish (○, ●).

Fig. 2 Change in the number of iron-containing granules in 300 μm of coelomic epithelium (pyloric caeca).

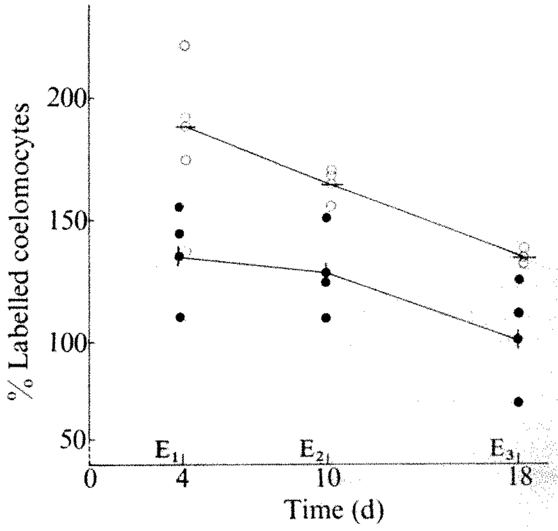

Table 1 Cellular labelling after intracoelomic injection of ferritin

Injected coeloms	Coelomocytes			Coelomic epithelia											Internal cavities of X and Y
				Digestive tract	Papulae	Ambulacral ampullae		Tube feet	Tiedemann bodies		Axial sinus		Axial organ (X)	Gastric haemal tufts (Y)	
	A	*B*	*C*	*A*	*A*	*A*	*B*	*B*	*A*	*B*	*A*	*C*	*C*	*A*	
Somatocoel	+	−	−	+	+	+	−	−	+	−	+	−	−	+	−
Hydrocoel	−	+	−	−	−	−	+	+	−	+	−	−	−	−	−
Axocoel	−	±*	+	−	−	−	−	−	−	±*	−	+	+	−	−

A, Somatocoelic; *B*, hydrocoelic; *C*, axocoelic.
*Passage through the dorsal orifice between stone canal and axial sinus.

Table 2 Evolution of total number of coelomocytes ($\times 10^6$)

Starfish	T	4 d → E$_1$			6 d → E$_2$			8 d → E$_3$		
	N	*N*	*M*	*V*	*N*	*M*	*V*	*N*	*M*	*V*
No. 1	180*	345	155	190	105	35	70	110	20	90
No. 2	31*	340	290	50	85	60	25	90	50	40

N, Number of coelomocytes; *M*, number of labelled coelomocytes; *V*, number of unlabelled coelomocytes.
*Estimation.

contained ferritin (Fig. 1), showing that they must originate from the somatocoelic epithelium. A piece of pyloric caecum was cut off after each bleeding and its peritoneal labelling was measured (number of iron particles per 300 μm epithelium): there was a progressive decrease of label (Fig. 2). A similar decrease of labelled free cells was found after each successive bleeding (Fig. 1). Thus, the labelled epithelial cells fall progressively into the body cavity.

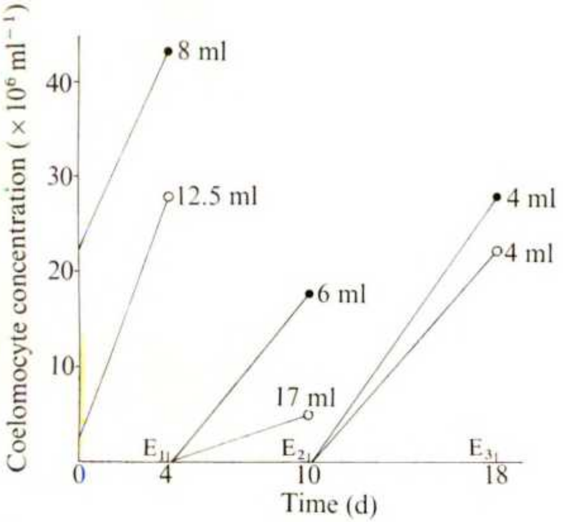

Fig. 3 Change of coelomocyte concentration. Total volume of coelomic fluid is marked for each line.

The introduction of foreign particles into the general coelom induced a considerable increase in the coelomocyte concentration (Fig. 3, T → E$_1$). Similarly, the concentration several days after bleedings E$_1$ and E$_2$ shows that the starfish can renew its coelomocytes remarkably quickly.

Table 2 shows the evolution of the total number of coelomocytes in the two starfish we used. Many of the new coelomocytes contained no iron. These cells obviously cannot have come from the division of circulating coelomocytes because the starfish had been bled. Furthermore, the results of injections demonstrate that no cells can have come from outside the coelomic cavity. All this suggests that the new coelomocytes in the general body cavity can have come only from the somatocoelic epithelium. This epithelium thus seems to offer great potential, as it could be the origin of both coelomocytes and digestive epithelial cells (in the case of regeneration)[15].

Observations of *A. rubens* larvae *in vivo* showed that the first free cells appear at the late bipinnaria stage (larvae ± 25 d old). At this stage the subdivision of the coelom is not yet complete and none of the presumed cytopoietic organs is present. Here again, the only possible source of coelomocytes is the coelomic epithelium.

Coelomic epithelial cells have a precisely known structure[16]. They are choanocyte-like cells, each apex of which is fitted with a long flagellum surrounded by a crown of microvilli. To form the coelomocytes, the cells detach themselves from the epithelium by stretching from their base (Fig. 4). Shortly after release, each cell loses its flagellum. This happens very fast, and it was by stimulating the formation of coelomocytes by injecting ferritin or Indian ink that we were able to observe it. This explains why flagellated cells had not previously been reported in the coelomic cavities of *A. rubens*[12].

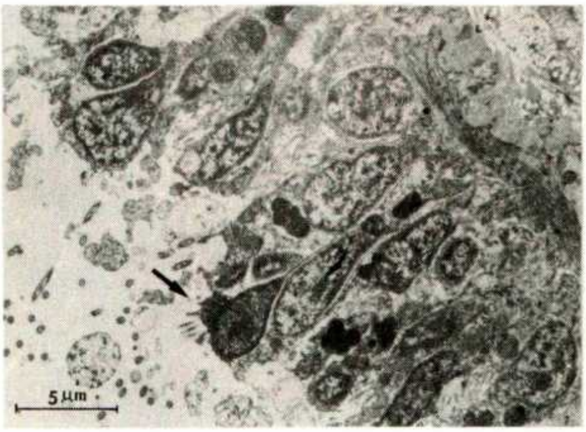

Fig. 4 Transformation of a coelomic epithelial cell into a coelomocyte (epithelium of the rectal caeca).

We conclude that the sole origin of coelomocytes in *A. rubens* is the coelomic epithelium and thus all organs lined with such epithelium can be considered to be cytopoietic.

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Electrophoretic evidence that selection reduces ecological overlap in marine limpets

CHARACTER divergence between sympatric, ecologically similar species has been attributed to selection which tends to increase the ecological difference between such species and to reduce the zone of their ecological overlap[1].

Evidence for this phenomenon is based solely on morphological characters which are presumably controlled by a number of genetic factors. There is, however, no documentation of divergence between sympatric species at the allelic level. During the course of a study of the population genetics of the intertidal limpet *Acmaea pelta,* I discovered what seemed to be an effect of coexistence with congeners on the frequencies of allozymes at a leucine aminopeptidase (*Lap*) locus. Populations of this highly eurytopic species from habitats offering widely different algal food and physical conditions, had similar frequencies of Lap allozymes, except from habitats where the populations were mixed with large populations of another acmaeid species (unpublished results).

A. pelta is one of fifteen intertidal species of its genus that are sympatric along the rocky shores of central California. A few of these species are rigidly stenotopic obligates of a single plant species, but most are eurytopic species that overlap considerably in their vertical distribution as well as in their utilisation of algal food[2,3]. A further study of the *Lap* loci of nine species of *Acmaea* using starch gel electrophoresis showed that each species has one *Lap* locus that gives a gel pattern of either two or four bands per individual. The pattern may be explained by proposing two polymeric enzymes which share a common polymorphic subunit as well as having different monomeric subunits[4]. Thus homozygotes show one band pair and heterozygotes two. This genetic interpretation of the patterns is supported by the fact that in none of the samples did the zygotic frequencies differ significantly from Hardy–Weinberg equilibrium, assuming two Mendelian alleles per animal. A total of eight band pairs were found each of which corresponded in mobility to one of the pairs found in *A. pelta.* The inferred alleles responsible for the mobility of these band pairs are designated $Lap^{0.19}$, $Lap^{0.46}$, $Lap^{0.73}$, $Lap^{1.00}$, $Lap^{1.24}$, $Lap^{1.54}$, $Lap^{1.81}$ and $Lap^{2.08}$, in order of increasing anodal mobility, with the most frequent allele of *A. pelta* as standard. Each species possesses two or more of these alleles. Figure 1 shows the frequencies of these alleles in six of the eurytopic species that commonly occur in mixed acmaeid assemblages. All of these species have different distributions of *Lap* alleles.

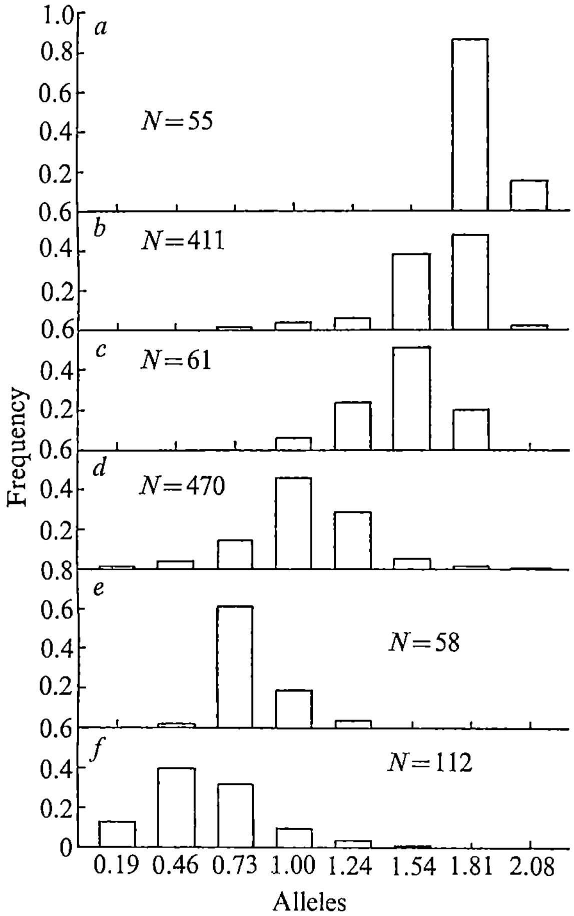


Fig. 1 *Lap* allele frequency distributions of six eurytopic species of the genus *Acmaea. a, A. persona, b, A. digitalis; c, A. scutum; d, A. pelta; e, A. strigatella; f, A. scabra.* Animals were held in running seawater for <24 h after capture. Extracts were made from live tissue, or from animals frozen intact and stored for less than two weeks at −20 °C. Repeated electrophoresis of individual extracts shows that storage for over a month at −20 °C results in a slight loss of Lap activity, but no change in electrophoretic pattern. The hepatopancreas of each limpet was removed and homogenised in an equal volume of gel buffer. After centrifugation, the extract was absorbed with 5-mm squares of Whatman 3MM filter paper. The squares were inserted into Electrostarch gels cast in moulds described by Smithies[5]. Gels were prepared and stained according to Brewer[6], except: 4% (w/v) sucrose was added to the gel; 0.32 M sodium citrate at *p*H 7.1 was used for electrode buffer, and a constant current of 10 mA was applied for 6 h. This method produced clear zymograms with good resolution of all allelic variants. Internal standardisations of mobility variants were carried out by including *A. pelta* individuals of known genotype on each gel. *N*, number of animals.

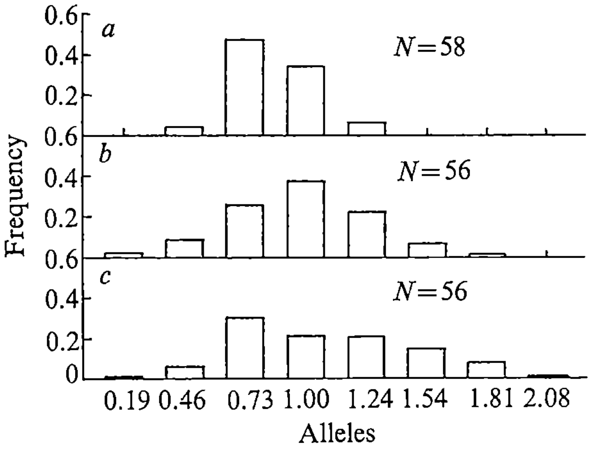


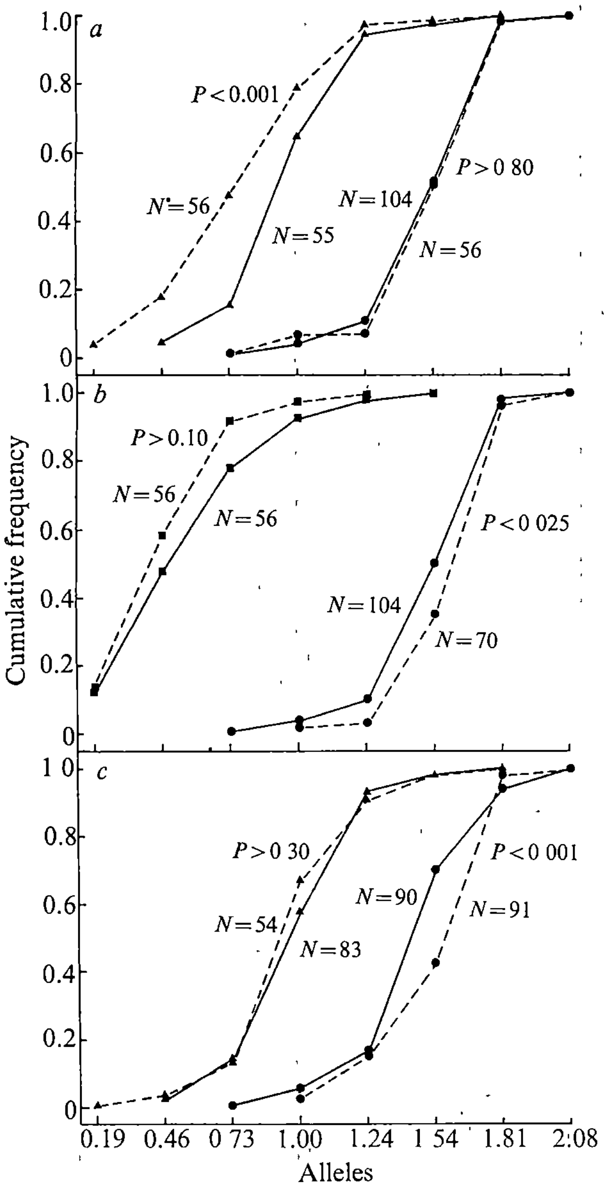
Fig. 2 *Lap* allele frequency distributions for three specialised species of the genus *Acmaea. a, A. instabilis; b, A. insessa; c, A. asmi.*

A. pelta is the most eurytopic of these species and coexists, at times, with all species in Fig. 1 except *A. persona. A. scutum* often coexists with *A. pelta* in the mid-interdial zone. Both here and at high interdial zones *A. scabra* and *A. digitalis* often occur in close association; either of these species can coexist with *A. persona* in the upper reaches of their vertical ranges. Lower down, either can coexist with *A. strigatella,* although *A digitalis–A. strigatella* associations are more common than *A. scabra–A. strigatella* associations. The *A. scabra–A. digitalis* association has been studied experimentally by Haven[7] and constitutes the only documented instance of interspecific acmaeid competition.

Since coexistence of ecologically similar species is likely to result in selection that will cause changes at many gene loci, the operation of this selection on closely related species that share identical alleles may have resulted in the species differences shown in Fig. 1. A precedent for the selective importance of the charge on molluscan Lap allozymes has been reported by Koehn and Mitton[8]. If the allelic differences among species that commonly coexist are instances of divergence resulting from selection to reduce ecological overlap, then a primitive or optimal distribution of alleles should be preserved in species which, in spite of their sympatry with others, do not compete. Several of the Californian acmaeids should escape competition since, as theorised by MacArthur and Levins[9], narrowly specialised species do not compete for those resources for which they are specialised. Three of these species are *A. instabilis*, an epizoite on Laminarian kelps, *A. insessa*, an epizoite on the kelp *Egregia menziesii*, and *A. asmi*, an epizoite on living shells of the snail *Tegula funebralis*. The distributions of *Lap* alleles in these three species tend to be broad, and all three have similar central tendencies (Fig. 2).

All eurytopic acmaeids that occur in mixed assemblages are also found in unispecific populations in some habitats. According to my interpretation of the above evidence, selection should operate only in habitats where species coexist, and there the interspecific similarity at the *Lap* loci should be a minimum. In order to test this prediction, habitats along 2 km of coast at Pigeon Point, San Mateo County, California, and along 200 m of coast at Pacific Grove, Monterey County, California, were chosen on the basis of their acmaeid assemblages. At each location samples were taken from habitats that supported unispecific populations of *A. pelta* or *A. digitalis*; samples of both species were also collected from a habitat that supported mixed populations of *A. pelta* and *A. digitalis*. In addition, at Pigeon Point, a sample of *A. scabra* from a unispecific population, and samples of *A. scabra* and *A. digitalis* from mixed populations, were collected. The results of the Lap allozyme analyses of these samples are shown in Fig. 3. In all instances, allelic distributions of coexisting populations differed more than the distributions of unispecific populations of the same species from the same location. The allelic distribution of only one species of each coexisting pair shifted in the predicted direction by a statistically significant ($P<0.05$) amount using a one-tailed Kolmogorov–Smirov two-sample test[10]. At Pigeon Point, where *A. digitalis* was twice as numerous as *A. pelta* in their shared habitat, the allelic distribution of *A. pelta* was affected (Fig. 3*a*), but that of *A. digitalis* was displaced at Pacific Grove, where it was mixed with about an equal number of *A pelta* (Fig. 3*c*). This agrees with a model of character displacement at one locus proposed by Crozier[11], which predicts that competition will cause displacement in both of a pair of sympatric species only in a narrow range of relative reproductive rates, and otherwise displacement will occur only in the more slowly reproducing species.

Fig. 3 Cumulative frequency diagrams of *Lap* alleles of unispecific (——) and coexisting (– – – –) populations of limpets. ▲, *A. pelta;* ●, *A. digitalis;* ■, *A. scabra* *a* and *b*, Pigeon Point, San Mateo County, California; *c*, Pacific Grove, California. *P* derives from the Kolmogorov–Smirov test of the H_1 that the allelic distribution of the coexisting population is not shifted in the predicted direction. *N*, number of animals.



The selection mechanism responsible for the observed genetic displacement is not known for certain. The possibility of selective settling of planktonic larvae of these limpets cannot be ruled out. Post-larval migration over short distances can be shown to be important in the distribution of *A. digitalis* of different shell patterns to appropriately coloured substrates[12]. Migration of settled acmaeids is, however, limited by their inability to cross sandy areas[13], which would have made post-larval migration between habitats unlikely in most of the cases I studied. Differential survival may be the agent, but my data show no significant correlation between size and genotype. This may mean that selection operates primarily on limpets too small for electrophoretic analysis.

Allelic accommodation in apparent response to competition has not been reported before, and, with acmaeids, should make possible the experimental study of the genetics involved in phenomena such as character displacement and niche diversification.

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Preferential pollination of yellow-flowered morphs of *Raphanus raphanistrum* by *Pieris* and *Eristalis* spp.

COROLLA-COLOUR polymorphisms occur in wild populations of many angiosperms, but little is known of the selective factors that maintain them. Valentine has suggested that yellow/white corolla-colour polymorphisms may be adaptively neutral[1]. I have observed extremely strong discrimination by some insect pollinators between the yellow and white corolla-colour forms of polymorphic wild radish. These observations, which are described below, indicate that corolla-colour differences are of adaptive importance in this and similar cases.

I have investigated the behaviour of pollinating insects visiting the flowers of wild radish (*Raphanus raphanistrum* L.), a self-incompatible annual[2] in which mixed populations of white-flowered and yellow-flowered morphs with sharply different corolla reflectance spectra (Fig. 1) are common in southern Britain. These forms differ only in a single allele, and no other characters have been observed to be associated with the difference in flower colour. Pollinator behaviour was observed in five polymorphic populations in central England and southern Wales during the summer of 1975. Bumble bees (*Bombus* spp.) and sometimes honey bees (*Apis mellifera*) were the chief pollinators, but in all populations *Pieris* spp., especially *P. rapae* (Lepidoptera, Pieridae) were important, often making 10–15% of the total number of visits to *R. raphanistrum*. *Eristalis* spp. (Diptera, Syrphidae) were minor pollinators, comprising up to ~5% of the visitors to *R. raphanistrum*.

Pieris spp. and *Eristalis* spp. showed a strong or very strong preference for the yellow-flowered morph of *R. raphanistrum* in four populations in which the frequencies of the yellow morphs ranged from 7.3 to 60.8% (Table 1). At the Sketty site, for example, where the frequency of the yellow-flowered morph was 60.8%, 307 visits by female *P. rapae* to *R. raphanistrum* were observed; 306 of these visits were to yellow flowers and only one to a white flower. *P. napi* and *P. brassicae* showed an equally strong preference for yellow-flowered *R. raphanistrum* at the same site. At this site, and at Brownhills and Beenham, *R. raphanistrum* was abundant, with yellow-flowered and white-flowered morphs closely intermingled in a mixed stand of field weeds. The Singleton population, which was the only artificial population, consisted of well-spaced rows of F_2 plants segregating at random for yellow or white flower colour. Although the frequency of yellow-flowered plants was only 27.3% in this population, the proportion of visits made to yellow flowers by *P. rapae* was higher than at the Brownhills site (42.3% yellows), and the proportion of visits made to yellow flowers by *E. arbustorum* was higher than at any other site, perhaps as a result of the spatial separation of individual plants at Singleton.

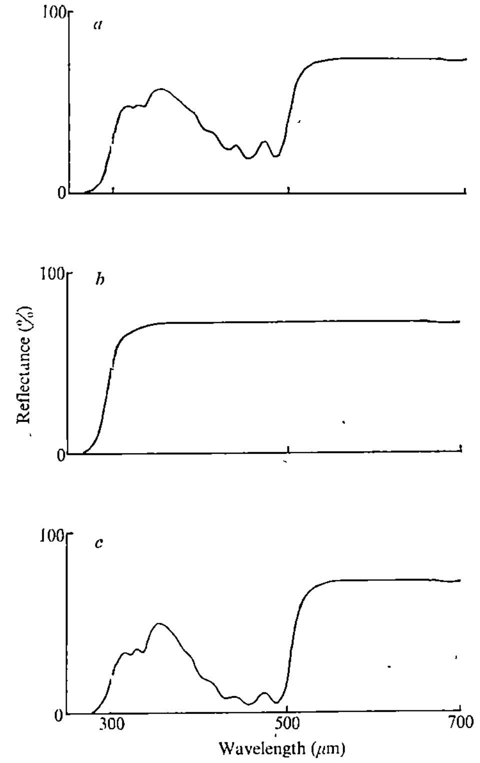

Fig. 1 Reflectance spectra of petals. Measurements were made using a Unicam SP 8000 recording spectrophotometer fitted with an SP 890 diffuse reflectance unit. *a*, *R. raphanistrum*, yellow morph; *b*, *R raphanistrum*, white morph; *c*, *Sinapis arvensis*.

The Landimore population differed from the others both in the low frequency of the yellow morph (0.8%) and in the occurrence of abundant *Sinapis arvensis* L., a crucifer with yellow flowers which resemble those of *R. raphanistrum* and have a closely similar reflectance spectrum to that of yellow *R. raphanistrum* (Fig. 1). *E. arbustorum*, *E. tenax* and other *Eristalis* spp. were abundant on *S. arvensis* flowers at this site, but did not visit *R. raphanistrum*. *P. rapae* and *P. napi* visited white-flowered *R. raphanistrum* and *S. arvensis* with approximately equal frequency. Only two cases of apparently preferential visits to yellow-flowered *R. raphanistrum* were observed at Landimore; a male *P. rapae* was involved in each case. Most male *P. rapae*, and all *P. napi* and female *P. rapae*, did not seek yellow-flowered *R. raphanistrum*.

In all populations the distances that were flown by individuals of the *Pieris* spp. between visited plants were commonly one to several metres, and flights of 15–40 m between successively visited plants were not unusual, with total distances of at least 150 m being covered during a series of visits. Recognition and location of yellow-flowered plants seemed to be entirely visual. Differences in behaviour between different individuals of *P. rapae* were observed at

Table 1 Frequency of visits by pollinators to the yellow-flowered morph of *R. raphanistrum* in five polymorphic populations

Site	Frequency of yellow morphs in population (%)	% of total visits made to yellow flowers *P. rapae* Males	*P. rapae* Females	*P. napi*	*P. brassicae*	*E. arbustorum*	*E. tenax*
Sketty	60.8 (418)	94.5† (292)	99.7† (307)	99.1† (111)	100† (107)	89.0† (556)	87.7† (235)
Brownhills	42.3 (130)	89.8† (303)	81.1† (169)	ND	ND	66.0* (47)	70.7† (75)
Singleton	27.4 (73)	92.3† (1,769)	89.3† (131)	93.7† (142)	ND	96.0† (569)	ND
Beenham	7.3 (303)	27.4† (446)	25.8† (190)	ND	ND	ND	40.8† (49)
Landimore	0.8 (240)	4.8† (268)	0.0 (233)	0.0 (470)	ND	ND	ND

The size of each sample is shown in parentheses. Significant differences from the population morph frequencies are indicated: $^*P<0.01$; $^\dagger P<0.001$. The grid references of the sites are as follows: Sketty, SS 623921; Singleton, SS 629917; Brownhills, SK 068052; Beenham, SU 592690; Landimore, SS 473926. ND, not done.

all sites but were most obvious at the Beenham site, where less than half of the individuals of both sexes sought yellow-flowered *R. raphanistrum*. In the natural populations, the strength of the preference that *P. rapae* showed for the yellow morph clearly decreased with decreasing frequency of the yellow morph. Pollinator discrimination of this type may have a role in determining the balance of the flower-colour morphs in *R. raphanistrum*, which shows both geographic clinal variation and local mosaic differentiation in flower-colour morph frequency. In Britain, the white morph predominates in polymorphic populations in southern and eastern England, but the yellow morph predominates or forms monomorphic populations in western and northern Britain.

There have been few investigations of pollinator behaviour in plant populations with flower-colour polymorphism, and no other case has been described in which pollinators discriminated so strongly between flower-colour forms of a wild species. Levin[3] found differences in attractiveness of about 1 : 1.49 between the least attractive (coral) and the most attractive (lavender) colour-forms of *Phlox drummondii* Hook. in artificial populations of mixed cultivars; he used pollen-load on the stigma as a measure of attractiveness, but did not observe the behaviour of pollinator species (mainly Lepidoptera) directly. Mogford[4] observed that some species of *Bombus* discriminated between purple and white flower-colour morphs of *Cirsium palustre* (L.) Scop. in some mixed populations; the greatest discrimination was shown by *B. lapponicus*, which made 88.4% of its visits to the white morph in a population with only 64.0% of white morphs, but discrimination was not consistent. The preference shown by *Pieris* spp. for the yellow morphs seems to conflict with the observations of Ilse[5] and Bennett[6], who reported preference for blue, violet, purple and white colours and relative avoidance of yellow flowers of various types by *Pieris* spp. The reflectance spectrum of yellow *R. raphanistrum*, however, shows a complex pattern of reflectance in the near ultraviolet between 275 and 400 nm (Fig. 1) and is thus bee purple[7] to bees and other insects with trichromatic colour vision extending into the near ultraviolet. Work on the spectral sensitivity of the eye of the butterfly *Heliconius*[8] has suggested that *P. rapae*, which is certainly sensitive to near ultraviolet reflectance[9], has this type of colour vision, and similar work on the spectral sensitivity of *E. tenax*[10] suggests that *Eristalis* spp. may have a similar type of colour vision. Ilse and Bennett did not consider ultraviolet colour components. Ilse[11] investigated the behaviour of *E. tenax* in relation to flowers of different colours, and successfully trained *E. tenax* to prefer yellow flower models (of Ostwald yellow paper, probably lacking an ultraviolet component) to those of other colours. Obara[9] observed that male *P. rapae* strongly preferred yellow to white paper models of females in summer, but did not discriminate in autumn; the near ultraviolet component of the colour may have been important. I did not, however, observe any consistent difference between male and female *P. rapae* in preference for the yellow morphs of *R. raphanistrum*, and it seems likely that feeding behaviour rather than mating behaviour determines the flower-colour preferences of *Pieris* spp.

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Vegetative reproduction and close packing in a successional plant species

HERBACEOUS perennials with determinate shoots commonly produce new aerial stems by rhizomes, stolons or adventitious root buds[1–5]. The usual result is a more or less dense clone. Preliminary field studies were made of tightly packed clones of goldenrods (*Solidago* spp., Compositae), common in late herbaceous stages of succession in the eastern USA. These studies suggested orderly geometric patterns of rhizome production.

A geometric model for development of dense clones was devised (Fig. 1). It was assumed that an optimal pattern of stem production maximises rate of centrifugal spread, maintains sufficient stem density in already colonised sites to produce a closed canopy, and minimises total rhizome length (and thus, biomass and respiratory load). The model requires that most parental rhizomes produce two or more daughter rhizomes, and that most daughter rhizomes diverge from the direction of parental growth by 60°, with one growing to the left (−60°) and one to the right (+60°) of the parental line of growth. Such a pattern, by itself, produces frequent gaps in the clone. The model also requires that occasional daughter rhizomes continue in the direction of parental growth. The stems subsequently produced by these 'non-divergent' rhizomes reduce the number of gaps left by divergent (60°) rhizomes. They also increase the rate of clone expansion.

The model incorporates some redundancy—that is, in ideal conditions, more than one stem may be produced at

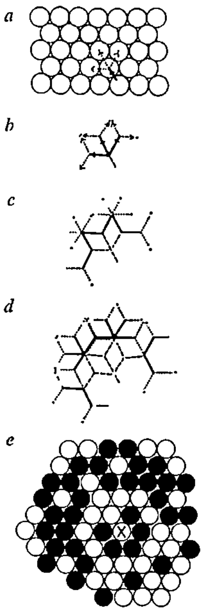

Fig. 1 Model of rhizome development during a 5-yr sequence. Dotted lines represent new rhizomes terminating in live aerial stems; solid lines represent 1-yr-old rhizomes; dashed lines represent rhizomes 2 yr old or more; *a*, parent rhizome and three daughter rhizomes; ○, represents a space to be filled by foliage from an aerial stem; *b*, same system 1 yr later; non-divergent (0°) daughter rhizomes are not produced in this and subsequent years; *c*, same system 2 yr later; *d*, same system 3 yr later; *e*, 4 yr later; ●, represents a space filled by foliage from one or more aerial stems; gaps could be filled by more frequent production of non-divergent rhizomes and by production of aerial stems directly from old stem bases; X, point of origin of the three daughter rhizomes shown in 1*a*.

a given point within the clone. In field conditions, rhizome mortality or damage, combined with environmental heterogeneity, would degrade optimal developmental patterns. Redundancy increases the probability that a given clone will maintain a closed canopy and maintain a rapid rate of centrifugal spread, in spite of environmental variables and developmental errors.

Clones of *Solidago canadensis* L. were used to test the model. *S. canadensis* is a common successional species which can form dense circular clones at least 2 m in diameter. Vegetative reproduction is by rhizomes, which produce aerial stems at their tips. Occasionally, new stems are produced directly from old stem bases. New rhizomes are produced only from stem bases. The study site was a field in eastern Pennsylvania (Lima, Delaware County) which was last ploughed 7 yr before the study. Slope was approximately 6–8% and the soil was a well-drained Glenelg channery silt loam[6]. Fifteen sod blocks were excavated from dense *S. canadensis* clones; each block was 0.7 m long, 0.4 m wide and 15–20 cm deep. Soil was washed off and rhizome geometry measured. Data included: the number of daughter rhizomes produced by each stem base; the rhizome length; and the angle formed by the base of each daughter rhizome and the straight line projected from the end of the parent rhizome. Rhizomes grew horizontally with little variation in depth below the surface. Thus, systems were described in a two-dimensional plane. All rhizomes had scale leaves oriented in the direction of growth, permitting separation of parent and daughter rhizomes.

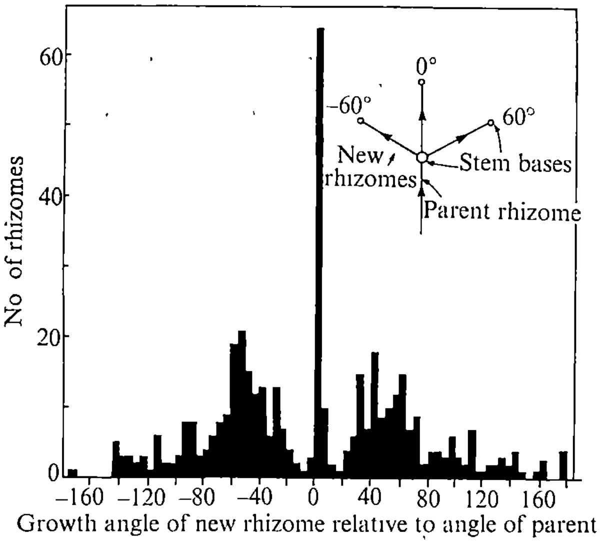

Fig. 2 Frequency distribution of rhizome growth angles. See text for explanation

Eighteen per cent of all rhizomes excavated continued in the direction of parental growth ('non-divergent'; Fig. 2). Any angle falling within 10° of parental growth direction was considered non-divergent. Of the remaining, divergent rhizomes, those that diverged to the left had a mean angle of −66.0±2.41° (mean±s.e.); those that diverged to the right had a mean angle of 67.6±2.95°. Mean number of daughter rhizomes per stem base was 2.43±0.104.

The data of Fig. 2 do not, in themselves, provide an adequate test of the model. Similar data could result from production of rhizomes diverging only to the right by one-half of all stem bases, and only to the left by the remaining stem bases. This pattern would not result in the predicted clone geometry of Fig. 1. Of the 106 stem bases that produced at least two diverging daughter rhizomes, 85.8% produced at least one rhizome diverging to the left and one diverging to the right. Of the 53 stem bases which produced at least two diverging rhizomes falling within 1 s.d. of one or both of the mean divergence angles (−66.0±32.98° and +67.6±37.97°), all had rhizomes falling within 1 s.d. of both left and right means. Thus, it was rare for all rhizomes produced at a given stem base to diverge in one direction.

Rhizome length could not be accurately determined for most systems because of breakage, because older rhizomes decay rapidly, and because the youngest rhizomes had not yet terminated in aerial stems. Mean linear distance between each stem base and the nearest adjacent stem base was 7.5±0.61 cm ($n=170$). It seems that rhizome length is frequently greater than this. New aerial stems are occasionally produced directly from old stem bases, increasing centripetal density. Some rhizomes grow at angles much greater than 60°, further increasing stem density in older portions of the clone.

Mean rhizome angles correspond closely to the model, suggesting some selection pressure for geometrical order. There is considerable variability in growth angle and rhizome frequency, resulting in breakdown of clone symmetry. The high degree of redundancy in the system, however, seems to compensate for this variability, resulting in the commonly observed pattern of closely packed aerial stems.

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Complementation of *ts* mutants by a herpes simplex virus *ts*-transformed cell line

THE first evidence that human herpesvirus could transform cultured cells was provided by Munyon *et al.*[1] who showed that the thymidine kinase activity of herpes simplex could be expressed in mouse-derived L cells lacking this enzyme (LTK^- cells). The oncogenic potential of human herpes virus was demonstrated by Duff and Rapp[2,3] who showed that hamster embryo cells transformed by ultraviolet-inactivated herpes simplex virus (HSV) were oncogenic in newborn hamsters. Further evidence of HSV transformation has been described in cultured human, rat and mouse cells[4–7]. The exact role played by HSV in transformation of primary embryo cells and the amount of information (if any) required to maintain the transformed state is not known. Temperature-sensitive (*ts*) mutants of HSV-2, HG 52 have been shown to complement each other[8]. These *ts* mutants may be used in complementation tests together with rat embryo cells transformed by another *ts* mutant to demonstrate the expression of herpes information in the transformed line. We report here evidence of such complementation between an HSV-2 *ts* mutant-transformed rat embryo line and three superinfecting HSV-2 *ts* mutants. This complementation suggests that this oncogenically

Table 1 Yield of virus from transformed and control cells infected with three complementing *ts* mutants of HSV-2

Mutant	No. of PFU in yield* after infection of cells for 24 h at 38.5 °C Control rat embryo cells	RE 1-transformed cells
ts 10	9	432
ts 10	2	298
ts 10	0	14
ts 3	3	210
ts 3	45	416
ts 3	272	578
ts 4	10	300†
ts 4	0	4†
ts 4	2	30†
ts 1‡	0	0
ts 1	2	4
ts 1	404	309
ts 1	0	0
ts 1	0	0

*Plaques obtained on titration at 31 °C: no plaques were found on titration at 38.5 °C

†Plaques obtained by infection of RE 1 cells by *ts* 4 were consistently larger than those on control plates.

‡Infection of transformed and control cells by *ts* 1, the mutant used for the original transformation of RE 1, was included as a control of the specificity of the complementation.

transformed line can express herpes information sufficient to complement at least three genes.

The transformation of rat embryo cells by *ts* mutants of HSV has been described[9,10]. Lines of HSV-transformed cells produced by picking foci and passaging these cells in tissue culture, have been shown to express HSV information in immunofluorescence tests with herpes-specific antiserum. Some of the transformed lines have been shown to be tumorigenic in rats (ref. 10 and our unpublished data) and the tumour cells, cultured *in vitro* express HSV information as seen by immunofluorescence. Extracts of tumours and of transformed cells express herpes-specific thymidine kinase (our unpublished data). Serum from tumour-bearing animals contains neutralising antibody and also antibody to herpes-coded thymidine kinase activity. Kimura *et al.*[11] have reported that at 38 °C the growth of two *ts* mutants was significantly enhanced in cells transformed by ultraviolet-inactivated HSV-2.

The line of transformed cells used was the RE 1 line described before[9,10]. Control cells were hooded Lister rat embryo primary cells and "hood" cell lines derived from them. Unpublished experiments have shown that it is not possible to distinguish the RE 1 line from primary rat embryo cells or hood cells by growth curves or saturation density attained. BHK 21 cells were used to prepare stocks of mutant virus and also for titration of virus in the complementation tests.

Tests for complementation were carried out by comparing the yields of virus from transformed RE 1 and from rat embryo control cells. Monolayers of transformed cells and of rat embryo control cells were infected with a *ts* mutant at a multiplicity of infection of 5 PFU per cell. Duplicate plates were used throughout. After adsorption for 1 h at 37 °C the virus inoculum was removed, and the plates were washed twice with Eagle's medium, incubated for 30 min at 38.5 °C with 5% antiserum, washed twice again with Eagle's medium and then incubated in Eagle's medium with 5% calf serum at 38.5 °C for 24 h. The cells were then scraped into the medium, disrupted by sonication and 0.2-ml samples were shaken with a suspension of 6×10^6 BHK 21 cells for 45 min at 37 °C. These cells were then divided between two 50-mm Petri dishes containing Eagle's medium with 5% calf serum; one plate was incubated at 38.5 °C for 2 d and the other at 31 °C for 3 d. Plates were then stained with Giemsa stain and the number of plaques obtained from infection of the RE 1 cells by a *ts* mutant was compared with the number obtained from control cells infected with the same mutant. A yield from the RE 1 cells of more than twice the number of plaques obtained from the control cells was regarded as evidence of complementation of the mutant virus by the transformed cells.

Results obtained with the HSV-2 mutants, *ts* 3 *ts* 4 and *ts* 10 are shown in Table 1. In each case there was evidence of complementation of the mutants by the transformed cell line. We have shown by fluorescence that HSV-specific proteins are produced in rat embryo cells infected with *ts* mutants (unpublished data) at 38 °C. This indicates that neither uptake nor uncoating are blocked.

Yields from the transformed cells infected with *ts* 4 regularly produced larger plaques than the parallel yields of control cells infected with this mutant, a finding which is under investigation.

No significant increase in yield was seen when the transformed cells were infected with *ts* 1, the mutant originally used to transform the cells. The information expressed in RE 1 cells cannot complement the homologous *ts* 1 mutant. It is also implied that complementation was specific for the expression of HSV information in the transformed cell line. In addition, the mutants *ts* 2 *ts* 5, *ts* 6, *ts* 7, *ts* 8 and *ts* 9 were tested, in duplicate on at least three separate occasions, but none showed significant complementation (Table 2), indicating that the relevant genetic information to complement these mutants is either absent or not expressed in the RE 1 cells.

Virus from plates showing complementation was tested for the presence of recombinants by replating the yield at 38 5 °C, but no recombinants were detected. Control hood cells after 21 and 34 passages behaved like primary rat embryo cells when infected with *ts* mutants. Thus the complementation of HSV *ts* mutants by RE 1 cells is unlikely to be just a consequence of their multiple passages in culture.

The four mutants *ts* 1, 3, 4 and 10 complement each other in standard genetic complementation tests[8] and the mutations are therefore in different genes. The fact that cells transformed by *ts* 1 complement the three mutants, *ts* 3, 4 and 10, suggests that at least three HSV functions are expressed in the transformed cells. HSV-specified thymidine kinase activity is expressed in RE 1 cells (our

Table 2 Yield of virus from transformed and control cells infected with six non-complementing *ts* mutants of HSV-2

Mutant	No. of PFU in yield* after infection of cells for 24 h at 38.5 °C Control rat embryo cells	RE 1-transformed cells
ts 7	0	0
ts 7	0	0
ts 7	0	0
ts 5	0	0
ts 5	0	0
ts 5	0	0
ts 9	170	24†
ts 9	0	0
ts 9	0	0
ts 8	0	0
ts 8	0	0
ts 8‡	20	38
ts 2	0	0
ts 2	0	0
ts 2	0	0
ts 6‡	30	50
ts 6	0	0
ts 6	0	0

*Plaques obtained on titration at 31 °C: no plaques were found on titration at 38.5 °C.

†The very small plaques noted here have been seen in a similar experiment done previously.

‡Mutants 6 and 8 do grow somewhat better in RE 1-transformed cells but the results were not significant in this set of data.

unpublished data), but this TK activity is not likely to be relevant to the *ts* mutant complementation demonstrated here, both because it is not normally an essential function for HSV replication[12], and because it is produced in normal amounts by all four mutants at 38 °C (ref. 13). Both *ts* 3 and *ts* 4 are DNA-synthesis-positive mutants but *ts* 1 and *ts* 10 are DNA synthesis negative[14]. *ts* 10 is slightly defective in production of viral DNA polymerase at 38 °C whereas *ts* 1 produces somewhat larger amounts than wild-type virus at 38 °C (ref. 13). Polymerase production, however, is probably not the primary defect of *ts* 10 and it is not yet possible to identify the functions involved in the complementation reaction in transformed cells. Our work suggests that the transformed RE 1 cells express not only HSV-specific antigens detectable by fluorescence and HSV-specific thymidine kinase activity, but also the HSV genetic information defective in three different HSV *ts* mutants. Our results with seven other mutants show that a large proportion of HSV-specified information is not expressed in these cells.

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Lectin-mediated attachment of glycoprotein-bearing liposomes to cells

LIPOSOMES have been used as vehicles for transporting drugs or enzymes into cultured cells and into tissues[1–8]. Before the full potential of liposome delivery systems can be realised, however, means must be found to direct the drug or enzyme bearing particles to specific sites within the body. Some modulation of the *in vivo* distribution of liposomes can be achieved by altering the size or charge of the vesicles[9,10]. More precise control of the interaction of liposomes with cells, and their distribution *in vivo*, may, however, be possible through the introduction of specific macromolecular entities into the liposome membrane. As a preliminary step in this direction we have incorporated a macromolecular lectin receptor into liposome membranes and have examined the lectin-mediated attachment of such liposomes to cells *in vitro*.

Our choice as a lectin receptor was the major sialoglycoprotein of the human erythrocyte membrane[11]. This component was extracted and purified to homogeneity[12] as evidenced by polyacrylamide gel electrophoresis (Coomassie blue staining). The erythrocyte sialoglycoprotein bears receptor activity for the lectins from wheat germ (WGA) and from *Phaseolus vulgaris* (PHA), but does not have appreciable receptor activity for concanavalin A (con A)[12,13]. The sialoglycoprotein was incorporated into liposomes which were mainly of the unilamellar type as formed by the technique of Batzri and Korn[14]. Briefly, an ethanolic solution of egg phosphatidyl choline and cholesterol (2 : 1 molar ratio) was injected rapidly into a solution of sialoglycoprotein in phosphate buffer. The sialoglycoprotein-containing liposomes formed were separated from free sialoglycoprotein by ultrafiltration through an Amicon XM 100A membrane, or by filtration on Sepharose 4B. The

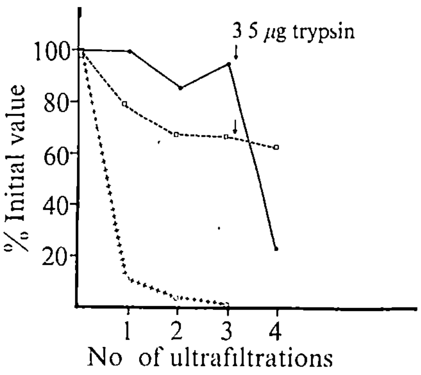

Fig. 1 Sialoglycoprotein liposomes were prepared by injecting 200 µl of an ethanolic solution of lipids (6 µmol egg phosphatidyl choline, 3 µmol cholesterol and a tracer amount of ^{3}H-dipalmitoyl phosphatidyl choline) into 4 ml of Dulbecco's phosphate buffer (*p*H 7.2). The buffer contained 100–500 µg of erythrocyte sialoglycoprotein and tracer amounts of ^{14}C-sialoglycoprotein; occasionally, low molecular weight solutes were included for trapping in the aqueous compartment of the liposomes. Free sialoglycoprotein and low molecular weight solutes were separated from liposome-bound material by repetitive ultrafiltration in Diaflow apparatus using an XM-100A membrane. Samples were repeatedly concentrated approximately tenfold by ultrafiltration and then diluted to the original volume. Aliquots were taken and sampled for radioactivity. At the indicated point the sialoglycoprotein liposomes were incubated with 3 5 µg ml^{-1} twice-crystallised trypsin for 30 min at 37 °C. ○, Free ^{14}C-sialoglycoprotein (SP); ●, liposome-bound sialoglycoprotein (SP), □, lipid-^{3}H-phosphatidylcholine (PC). The loss of ^{3}H-lipid during the first two ultrafiltrations is probably due to absorption of isotope on to hydrophobic sites in the membrane filter. It can be reduced by prewashing the filter with an albumin solution.

Table 1 Haptene inhibition of sialoglycoprotein liposome attachment to red cells

	Expt	WGA(%)	PHA (%)	RCA (%)
Lectin	I	100	100	100
	II	100	100	100
Lectin + haptene	I	47	91	0
Sugar	II	0	0	—
Lectin + non-haptene	I	106	107	125
Sugar	II	—	53	—
Lectin + post-treatment	I	105	—	0
W. haptene	II	99	25	—

Erythrocytes were treated with the indicated concentration of lectins washed and exposed to sialoglycoprotein liposomes for 2 h at 25 °C. In some cases appropriate haptene sugars (*N*-acetylglucosamine for WGA, *N*-acetylgalactosamine for PHA and galactose for RCA) or mannose as a non-haptene sugar were included in the reaction mixture. The samples were then processed as described in Fig. 2. Nonspecific attachment (in the absence of lectin pretreatment) was subtracted from the data. The results are expressed as the percentage of the effect produced by the lectin alone. The results represent the means of triplicate determinations. Experiment I, lectin concentrations were 50, 20 and 80 µg ml^{-1} for WGA, PHA and RCA, respectively. The haptene and non-haptene sugars were present at a final concentration of 50 mM. Experiment II, lectin concentrations were 100 and 20 µg ml^{-1} for WGA and PHA, respectively. The haptene and non-haptene sugars were present at a final concentration of 250 mM.

sialoglycoprotein from erythrocytes has previously been incorporated into artificial lipid membranes by several other workers[15–18].

The degree of incorporation and the ratio of protein to lipid in the liposome preparations were measured using ^{3}H-dipalmitoyl phosphatidyl choline and ^{14}C-sialoglycoprotein (labelled by reductive alkylation with ^{14}C-formaldehyde[19]), both of known specific activity. The sialoglycoprotein was readily incorporated into the liposomes at low protein–lipid ratios and somewhat less efficiently incorporated at higher ratios. Thus the percentage incorporation varied from 90 to 30% using 10–200 μg protein per mg phosphatidyl choline. Most of our preparations had a sialoglycoprotein–phosphatidyl choline weight ratio of 0.10–0.30, but higher ratios could also be achieved. Assuming a mean molecular weight 2×10^6 for unilamellar vesicles[20], and a molecular weight of 53,000 (ref. 12) for the sialoglycoprotein, then most of our preparations contained an average of 2–6 sialoglycoprotein molecules per vesicle. Much of the incorporated sialoglycoprotein seemed to be arrayed at the outer surface of the liposome since it could be cleaved from the vesicles by low doses of trypsin (Fig. 1*a*). In experiments using 3.5–75.0 μg ml^{-1} trypsin, from 72 to 87% of the sialoglycoprotein was cleaved from the vesicles as judged by release of radioisotope. It is unlikely that the protease penetrated into the vesicles in the conditions used since incubation with substantially higher doses (100 μg ml^{-1}) of trypsin failed to release any of the ^{3}H-sucrose sequestered within the aqueous compartment of the liposomes. These results contrast with those of MacDonald and MacDonald[17] who used another technique for preparing sialoglycoprotein liposomes and found only 50% of the incorporated protein exposed at the outer surface of the vesicles. We have not yet characterised the physical properties of the sialoglycoprotein liposomes in great detail; however, columns of Sepharose 4B largely retarded these liposomes yielding an elution profile typical of a preparation containing mainly small unilamellar vesicles[21].

Liposomes bearing sialoglycoproteins can participate in agglutination reactions mediated by appropriate lectins such as WGA. This can be in the form of self agglutination as first described by Redwood and Jansons (ref. 16 and Fig. 2*a*) or it can be mixed agglutination with lectin-coated cells such as erythrocytes (Fig. 2*b*). Both types of agglutination reaction exhibit apparent saturation and display a sigmoidal dependence on the amount of lectin used. The rate of lectin-mediated attachment of liposomes to cells is rapid at first and then slows substantially, with maximal attachment after 3–4 h at 25 °C (Fig. 2*c*). The mixed liposome–cell agglutination reaction seems slow compared with the reported kinetics of lectin-mediated agglutination of cells, where the reactions went to completion in about 20 min (ref. 22). The participation of the liposomes in lectin-mediated agglutination to cells is governed by the receptor specificities borne by the sialoglycoprotein. Thus WGA and especially PHA, which have high affinities for isolated sialoglycoprotein[12],

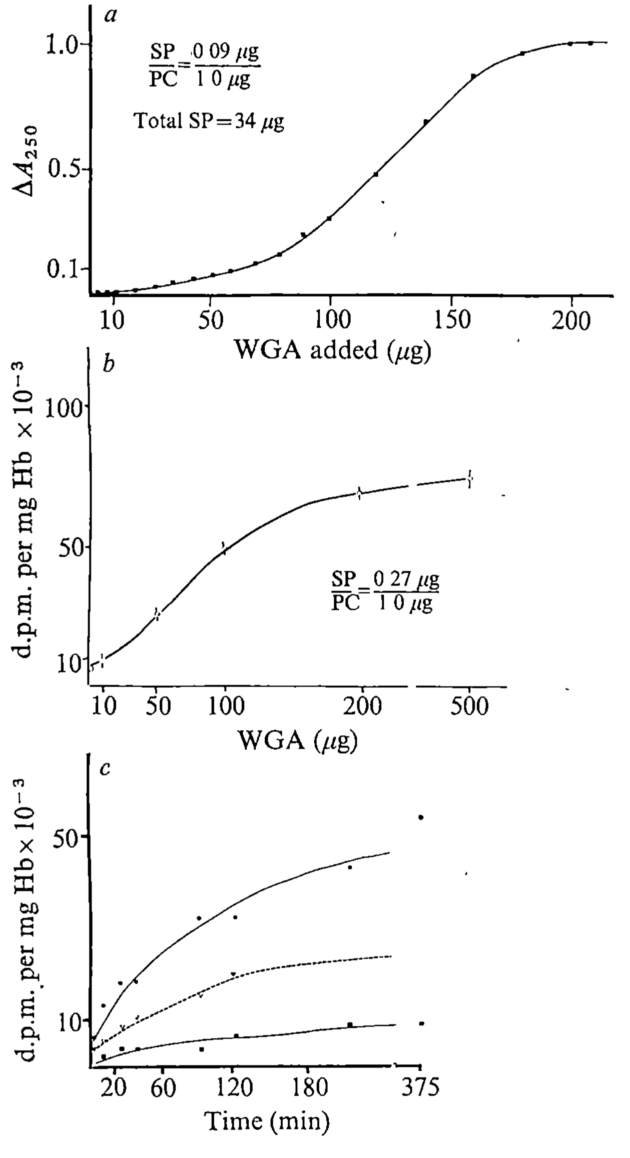

Fig. 2 *a*, Self agglutination of sialoglycoprotein liposomes. The agglutination of sialoglycoprotein liposomes was followed by light scattering measurements in a Beckman CII double-beam spectrophotometer. The absorbance of a sample cuvette containing sialoglycoprotein liposomes and WGA was compared with that of a reference cuvette containing an equal amount of sialoglycoprotein liposomes and WGA as well as 100 mM *N*-acetyl glucosamine. The difference in absorbance A_{250} was determined just after stirring both cuvettes and 5 min after each addition of WGA. The sialoglycoprotein (SP) – phosphatidyl choline (PC) ratio is indicated. Attachment of sialoglycoprotein liposomes to cells—experiments involving the binding of sialoglycoprotein liposomes to erythrocytes were conducted as follows. Aliquots (5 ml) of a 2% suspension of human red cells in Dulbecco's buffer were incubated with the indicated doses of lectins for 30 min at 4 °C. The lectin-coated cells were recovered by centrifugation and washed three times in buffer. Clumps of cells were dispersed by gentle pipetting Aliquots (2 ml) of lectin-coated red cells in buffer were incubated with 100 µl of sialoglycoprotein liposomes containing ^{3}H-dipalmitoyl phosphatidyl choline at 25 °C and usually for 120 min with constant mixing. The liposome–cell mixture was layered on 2 ml of a 10% albumin solution in buffer and the red cells were separated from unbound liposomes by sedimentation at 2,500 r.p.m. for 15 min. The cell pellet was dispersed and aliquots were taken for haemoglobin determination and for radiation counting. Efficiency corrections were made using a curve generated from samples quenched with whole blood. *b*, Dose dependence of WGA-mediated attachment of sialoglycoprotein liposomes to red cells at 25 °C. Abscissa, WGA (µg) used in pretreating red cells; ordinate, d.p.m. of ^{3}H-phosphatidyl choline per mg of haemoglobin. Mean ± s.e. shown ($n=3$). *c*, Kinetics of WGA-mediated attachment of sialoglycoprotein liposomes to red cells. Erythrocytes were treated with 100 µg of WGA in buffer, or with buffer only. The time course of attachment of sialoglycoprotein liposomes to cells at 25 °C was measured as above. ●, + WGA; ▽, + WGA + 50 mM *N*-acetylglucosamine; ■, − WGA. For these experiments wheat germ agglutinin (WGA) and con A were obtained from Sigma, crude PHA was from Difco RCA was prepared as described previously[30].

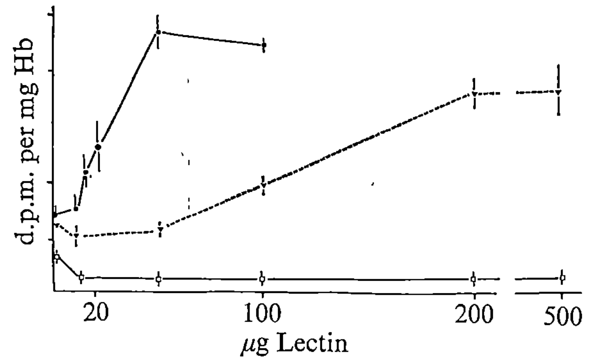

Fig. 3 Dose dependence of lectin-mediated attachment of sialoglycoprotein to erythrocytes at 25 °C. Protocol as in Fig 2*b*. Abscissa, lectin (μg) used in pretreatment of erythrocytes; ordinate, d.p.m of ^{3}H-phosphatidyl choline per μg of haemoglobin ●, PHA; ▼, RCA; □, con A. Mean ± s.e. shown (n=4).

are effective in promoting liposome attachment to cells, whereas con A, which does not interact with the sialoglycoprotein, fails to cause liposome–cell attachment (Fig. 3). A possible anomaly in our results seems to be the case of *Ricinus communis* agglutinin (RCA) which has been reported to interact only weakly with the isolated sialoglycoprotein[12], but which promotes strongly sialoglycoprotein liposome attachment to cells (Fig. 3). This discrepancy may be due to the presence of RCA receptor components in our sialoglycoprotein preparation which react poorly with Coomassie blue or with periodic acid–Schiff stains. RCA receptors from erythrocytes are known to have such characteristics and to overlap partially with the major sialoglycoprotein in gel electrophoresis[13]. As Table 1 shows, the presence of appropriate haptene sugars reduces the lectin-mediated attachment of sialoglycoprotein liposomes to cells. Only in the case of RCA, however, can the liposomes be fully released from the cell–liposome aggregate by post-treatment with the haptene sugar. It seems likely, in this case at least, that the liposomes are bound at the surface of the erythrocyte and are not internalised.

The maximal levels of lectin-mediated sialoglycoprotein liposome attachment to erythrocytes seen in Figs 2 and 3 represent the binding of 10–25 μg of phospholipid per mg of haemoglobin. By comparison, the normal human erythrocyte contains approximately 8.9 μg of phospholipid per mg of haemoglobin[23]; thus the liposomes bound to the erythrocytes by lectins comprise 2–3 times the total lipid of the red cell membrane. Since these liposomes probably possess a single bilayer membrane, our observations would suggest that, at maximal levels of attachment, the erythrocyte surface is largely covered by the bound vesicles.

Table 2 Efflux rates of $^{35}SO_4^{2-}$ from liposomes

	First hour	Second hour	Third hour
Sialoglycoprotein liposomes	11.8	11.4	9.8
Sialoglycoprotein liposomes + 100 μg ml^{-1} RCA	16.2	14.0	13.9
Sialoglycoprotein liposomes + 100 μg ml^{-1} RCA + 50 mM galactose	12.5	10.8	8.4
Sialoglycoprotein liposomes + 1 mg ml^{-1} RCA	26.6	18.0	12.9
Sialoglycoprotein liposomes + 1 mg ml^{-1} RCA + 50 mM galactose	16.5	13.8	11.8
Triton X–100 (0.5%)	77.3		

Sialoglycoprotein liposomes were prepared containing trapped $Na_2{}^{35}SO_4$ and the efflux rate at 25 °C of $^{35}SO_4^{2-}$ was measured as previously described[29]. Results are expressed as percentage efflux per h.

To assess the possibility that lectins disrupt the structure of sialoglycoprotein liposomes during aggregation reactions, we have studied the efflux of $^{35}SO_4^{2-}$ from such liposomes. Table 2 shows that the efflux rate of $^{35}SO_4^{2-}$ from sialoglycoprotein liposomes was approximately 10% per h, which is within the range commonly observed for anion fluxes in negatively charged liposomes[21]. The introduction of a lectin (RCA) at a concentration up to 1 mg ml^{-1} had less than a twofold effect on the efflux rate, and some of this effect may have been due to nonspecific protein–lipid interaction. These results suggest that lectin-mediated aggregation of sialoglycoprotein liposomes does not result in gross perturbation of the structure of the liposome membrane.

The SP liposome–lectin system described here may be applicable to investigation of the biochemical basis of lectin-mediated agglutination of cells. This phenomenon is a complex one, involving the number and affinity of lectin receptors, the mobility of receptors in the membrane and the interactions of receptors with active cytoplasmic elements[22,24,25]. In our system, several of these factors are subject to precise experimental manipulation. For example, the average receptor density per particle may be varied by altering the lipid–protein ratio during liposome formation. The receptor mobility may be controlled by preparing liposomes from synthetic phospholipids with known transition temperatures, followed by investigation of the agglutination reaction above or below the thermal transition. This approach may be complementary to studies using glutaraldehyde fixation to modify receptor mobility[26] since fixation may indirectly affect receptor mobility by altering intracellular elements such as microtubules or microfilaments, whereas alteration of the lipid composition of the SP liposome would directly modify membrane fluidity.

The system we have studied, namely the mixed aggregation of receptor-bearing liposomes and cells, may have some advantages over systems which use the self aggregation of liposomes[16,27]. Thus, we can examine the interaction of a well characterised receptor-bearing particle with undefined receptors on cells. The effects of manipulating cellular physiology on the aggregation reaction can also be studied.

We feel that our studies represent a step towards the development of liposomes which can be 'targeted' to specific cell types *in vitro* or *in vivo*. During the course of these studies Gregoriadis and Neerunjun[28] reported on the use of specific antibodies to 'target' drug bearing liposomes *in vitro*. Our endeavours and those from Gregoriadis' laboratory seem similar in intent although different in approach.

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Mycoplasma pneumoniae is a polyclonal B-cell activator

MYCOPLASMA PNEUMONIAE (MP) is an important causative agent of lower respiratory tract illness in man[1]. Lymphocytes from subjects who have experienced MP infection show a high degree of stimulation when cultured in the presence of MP antigen[2,3]. But high concentrations of MP antigen also stimulate, to some extent, lymphocytes from individuals without serological evidence of previous MP infection, suggesting that these subjects have either been infected with MP in the past or that MP also has a non-antigen-specific, mitogenic effect[2]. Several mitogens are known: the plant lectins phytohaemagglutinin (PHA) and concanavalin A, for example, have a mitogenic effect on T lymphocytes[4], and certain bacterial products, such as lipopolysaccharide (LPS) of *E. coli*[4] and a purified protein derivative of tuberculin (PPD) are mitogenic for mouse B cells[5] and induce polyclonal antibody synthesis in mouse spleen cells[4]. These substances are called polyclonal B-cell activators (PBA) (ref. 6). We now report that MP is mitogenic for mouse and guinea pig lymphocytes and induces polyclonal antibody formation in mouse spleen cells.

Cells were obtained from inbred C57BL mice, specific pathogen-free congenitally athymic Nu/Nu mice on a BALB/c background (Bomholtgaard, Ry, Denmark) and normal outbred guinea pigs. Spleen or thymus cells were cultured as described previously[7] in Eagle's medium in Eagle's solution, or in RPMI 1640 medium with added L-glutamine, penicillin and streptomycin. Unless otherwise stated, serum was omitted from the cultures. DNA synthesis was measured and antibody-secreting cells were counted as before[7]. Briefly, 5×10^5 cells in 0.2 ml were cultured per well in microplates, usually for 48 h with a 24-h pulse of 1 μCi ^{3}H-thymidine or an 18-h pulse of 0.05 μCi ^{14}C-thymidine, and collected in a multiple cell culture collector (Skatron A/S, Lierbyen, Norway). Radioactivity was measured in a liquid scintillation counter. To determine antibody secretion, 10^7 cells per ml per dish were cultured in plastic Petri dishes. Antibody-secreting cells were counted after 2 d of incubation in a modification of Jerne's haemolytic plaque assay, using as targets sheep red blood cells (SRBC), horse red blood cells (HRBC), or SRBC coupled with the following haptens or antigens: (4-hydroxy-3,5-dinitrophenyl) acetyl (NNP), trinitrophenyl (TNP), fluoresceinisothiocyanate (FITC)[7] or dextran[8].

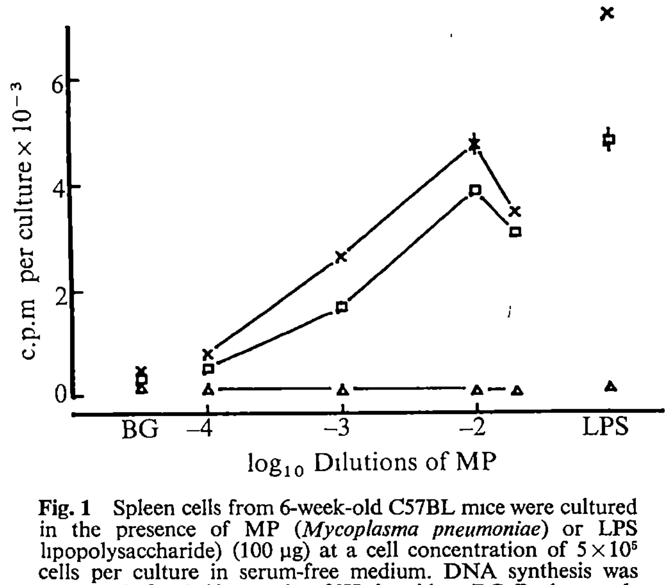

Fig. 1 Spleen cells from 6-week-old C57BL mice were cultured in the presence of MP (*Mycoplasma pneumoniae*) or LPS lipopolysaccharide) (100 μg) at a cell concentration of 5×10^5 cells per culture in serum-free medium. DNA synthesis was measured after 2 d by uptake of ^{3}H-thymidine. BG, Background; □, spleen cells; ×, spleen cells treated with carbonyl iron; △, thymus cells

Table 1 Response of spleen cells to various antigens

	PFC per culture ± s.e. BG	MP	LPS
NNP–SRBC	153±12	2,376±125	2,297±294
TNP–SRBC	177±7	1,773±104	2,290±215
FITC–SRBC	90±6	1,233±151	1,480±114
Dextran–SRBC	10±1	133±17	120±20
SRBC	20±6	113±9	137±9
HRBC	100±30	516±68	380±55
DNA synthesis (c.p.m. per culture)	5,236±276	25,697±881	33,940±1,060

Spleen cells from Nu/Nu mice were cultured in the presence of MP antigen (diluted 1/100) or LPS (100 μg) at a cell concentration of 5×10^6 cells per culture in medium with 5% foetal calf serum. Polyclonal antibody synthesis was measured after 2 d by detecting numbers of PFC to SRBC, HRBC and SRBC coupled with NNP, TNP, FITC or dextran. Determination of DNA synthesis was done in microcultures (5×10^5 cells per culture) pulsed for 24 h with 1 μCi ^{3}H-thymidine. BG, Background.

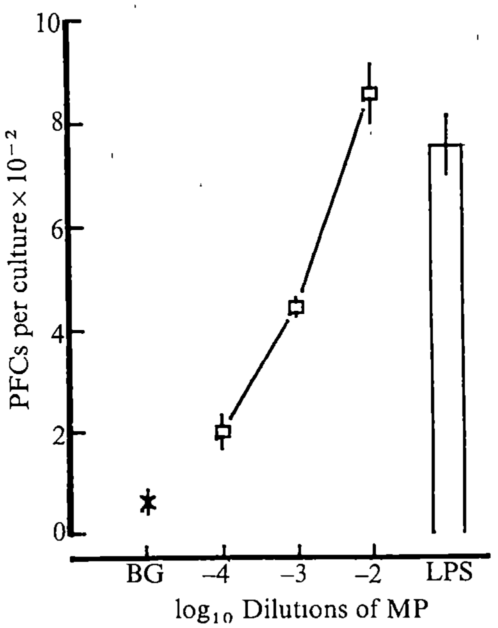

Fig. 2 Spleen cells from 6-week-old C57BL mice were cultured in the presence of MP or LPS (100 μg) at a cell concentration of 10^7 cells per culture, in serum-free medium. Polyclonal antibody synthesis was measured after 2 d by detecting numbers of PFC against SRBC coupled with NNP.

MP antigen was prepared as before[2] from organisms grown in broth on a glass surface. A concentrate of MP organisms was ultrasonicated and heated at 56 °C for 30 min to kill all viable organisms. The protein concentration of undiluted antigen was $2.5\ \mathrm{mg\ ml^{-1}}$. LPS (obtained from Dr T. Holme, Stockholm) was extracted from *E. coli* 055 : B5 by the phenol water method. PPD (tuberculin RT 32) was obtained from Statens Seruminstitut, Copenhagen.

After addition of MP, spleen cells from normal mice responded with increased thymidine uptake (Fig. 1) as well as antibody secretion (Fig. 2). The peak of activation occurred on days 2 and 3 (data not shown). Removal of adherent cells only enhanced DNA synthesis (Fig. 1), and normal thymocytes did not respond. As the medium used for growing the MP organisms had no effect on either DNA synthesis or antibody secretion, we concluded that MP acts as a polyclonal activator for mouse spleen cells.

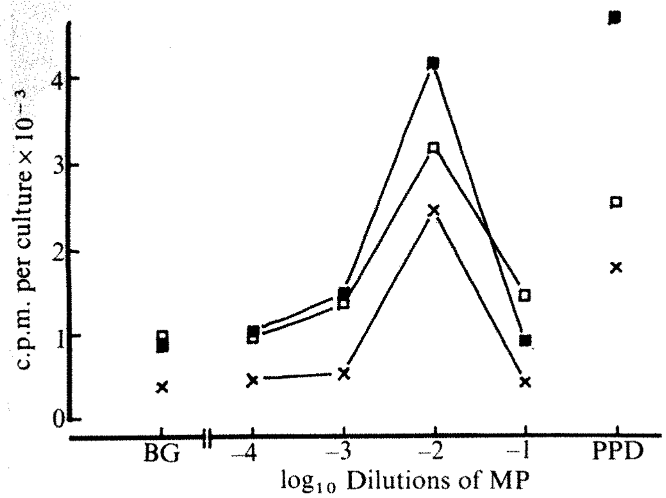

Fig. 3 Spleen cells from each of three guinea pigs were cultured in the presence of MP or PPD (100 μg) at a cell concentration of 5×10^5 cells per culture in serum-free medium. DNA synthesis was measured after 2 d by the uptake of ^{14}C-thymidine. Each symbol represents the mean of triplicates of cells from one animal.

Adherent cells were not necessary for activation, and thymocytes did not respond, indicating that MP was a polyclonal B-cell activator. Indeed, spleen cells from Nu/Nu mice responded both by increased thymidine uptake and by producing increased numbers of antibody-secreting cells to various antigens (Table 1).

The multispecificity of the MP-induced antibody response and the fact that cells from pathogen-free mice responded makes it unlikely that stimulation was due to a previous *in vivo* infection by a microorganism cross reacting with MP antigens.

MP also stimulated DNA synthesis in spleen cells from normal guinea pigs (Fig. 3). Some animals showed a stronger response to MP than to PPD, whereas others responded better to PPD. Experimental MP infection in guinea pigs serves as a model for mycoplasma infection in man[9], and so the guinea pig is a suitable animal for studies of the mitogenicity of MP *in vivo*.

An animal mycoplasma, *M. pulmonis*, has been shown to stimulate rat lymphocytes nonspecifically[10]. It is not known whether the responding cells were B or T lymphocytes. The stimulating factor of this mycoplasma was heat labile, in contrast to the mitogenic component of MP.

The results reported here do not provide information about the chemical nature of the MP component responsible for the mitogenic effect. The antigens eliciting specific antibodies to MP are located mainly in the mycoplasma cell membrane, which consists essentially of a lipid bilayer with proteins[11]. The main serologically active MP components are glycolipids with haptenic properties[12,13]. Previous studies have shown that a lipid extract of MP, reacting with MP antisera in the complement-fixation test, did not stimulate sensitised or non-sensitised human peripheral blood lymphocytes[2]. Further studies are necessary to elucidate the relationship between antigenicity and mitogenicity of various MP components.

Antibodies synthesised by mitogenically activated cells *in vitro* are most often of the IgM class[6]. Since MP infection in man is often followed by an increase in serum IgM, which is only partly due to specific anti-MP antibodies[14], we suggest that this increase could in part be the result of MP-induced mitogenic activation of B cells. The fact that IgM antibodies directed to autoantigens are found after MP infection[1,15] supports our suggestion. Mitogenicity of MP towards human lymphocytes has still to be firmly established, however.

In summary, our data show that MP, an important pathogen in man, is a polyclonal activator for mouse B lymphocytes and has a mitogenic effect on guinea pig spleen cells *in vitro*. The ability of MP to activate B cells polyclonally might influence the antibody response of patients and experimental animals infected with this microorganism. Finally, the fact that almost all substances which act as polyclonal B-cell activators are derived from microorganisms might indicate the importance of mitogenic activation for an effective immune defence[16].

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Purported difference between human T- and B-cell surface morphology is an artefact

BECAUSE of the previous inability to detect differences in lymphocyte morphology by light or transmission electron microscopy[1], reports that B and T lymphocytes examined with scanning electron microscopy (SEM) could be identified by their distinctive surface morphology have been met with interest[2–6]. In these studies, most 'villous' cells were identified as B lymphocytes and T lymphocytes were described as 'relatively smooth'. Some investigators have reported that T lymphocytes are more villous than B lymphocytes[7–9]; others, however, have not detected a difference in surface form among human[10,11], or murine lymphocytes[12–14]. The present study demonstrates that the

Table 1 Induction of smooth normal and leukaemic lymphocytes by an SEM preparative technique

	% Smooth cells	
	Fixed in suspension[15]	Fixed after aspiration filtration[2-5]
Normal peripheral blood*	0.7±0.3	94.5±0.7
Sezary's syndrome (T-cell leukaemia)†	1.6±0.3	81.2±4.5
CLL (B-cell leukaemia)†	2.0±0.3	85.8±5.4

*Mean ± s.e.; 4 experiments; 500 cells evaluated in triplicate samples per experiment (total $n = 6{,}000$).

†Mean ± s.e.; 500 cells evaluated in triplicate samples per patient ($n = 1{,}500$ per patient).

collection and fixation technique used in those studies that initially described the dichotomy in lymphocyte SEM morphology[2-5], artefactually produces smooth cells.

The surface morphology of normal mononuclear leukocytes prepared for SEM by the aspiration filtration technique[2-5] was contrasted with that of cells from parallel samples fixed in suspension (Table 1). When cells were fixed in suspension and processed on poly-L-lysine-coated glass coverslips, more than 97% of the cells were retained for SEM observation[15]. Approximately 93% were covered uniformly with variable numbers of short microvilli, 6% were ruffled, and less than 1% was smooth (Table 1, Fig. 1*a*). When parallel cell suspensions were aspirated live on to the filters with subsequent fixation, however, approximately 95% of the leukocytes appeared relatively smooth (Table 1).

Leukaemic cells of B- or T-lymphocytic origin were analysed in the same manner. Ninety-nine per cent of the blood lymphocytes from a patient with Sezary's syndrome were considered to be T lymphocytes on the basis of the formation of spontaneous E rosettes with sheep erythrocytes and the failure to stain with fluoresceinated anti-human immunoglobulin. Approximately 80% of the purified lymphocytes from a chronic lymphocytic leukaemic (CLL) patient were B lymphocytes, by the criteria of the presence of surface immunoglobulin and the binding of heat-aggregated immunoglobulin and antigen–antibody complexes, and 99.5% failed to form E rosettes. Although 98% of the lymphocytes from these patients with leukaemias of B- or T-cell origin were villous or ruffled when fixed in suspension, 81% of the T-leukaemic cells and 86% of the CLL cells appeared smooth when aspirated live on to filters (Table 1 and Fig. 1*b*, *c*, *e* and *f*).

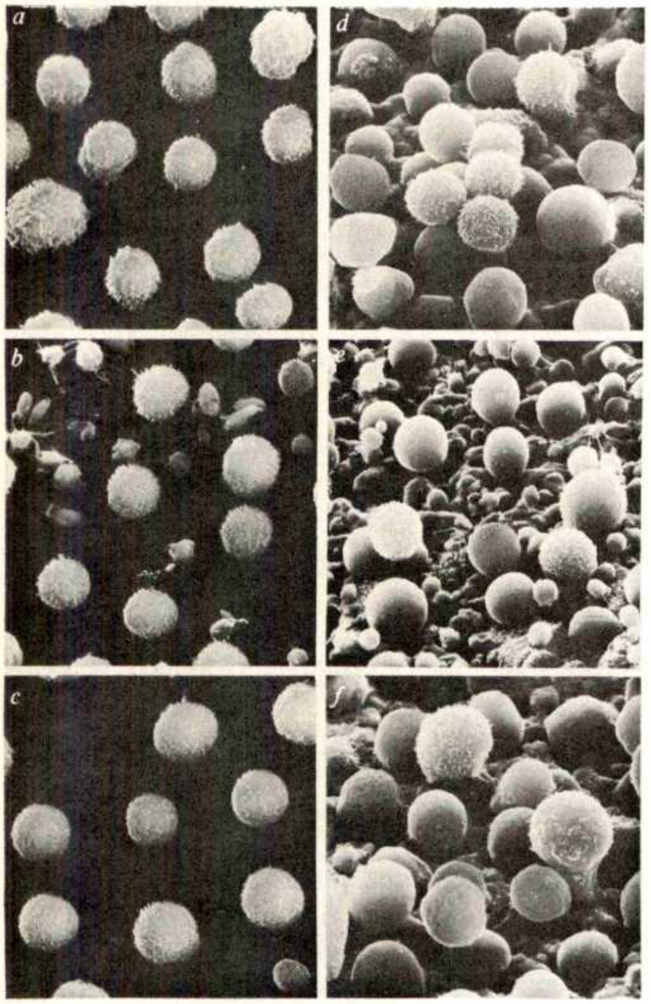

Fig. 1 Human peripheral mononuclear leukocytes were obtained from the defibrinated fresh blood of four normal donors by Ficoll–Hypaque density gradient centrifugation[16]. Differential counts indicated that on the basis of morphology 3% of the white cells were polymorphonuclear lymphocytes, 12% monocytes, and the remaining 85% lymphocytes. Leukaemic lymphocytes were obtained from the heparinised blood of a patient with CLL and by leukophoresis from a patient with Sezary's syndrome, and processed as outlined for normal human peripheral blood. 95–98% of the cells in these populations were lymphocytes. Surface marker studies were carried out on these purified leukaemic lymphocyte populations. E rosettes were carried out by a method published previously[17]. Surface immunoglobulin and aggregate binding and antigen–antibody complex binding were detected as described previously[18,19]. Cell viability as determined by Trypan blue dye exclusion was greater than 95% for all preparations. Cells from each donor were processed for SEM by each of two methods. First, cells were fixed in suspension for 24–48 h at 22 °C by centrifuging 2×10^6–8×10^6 cells at 200*g* for 10 min, resuspending the cell pellet in 1.0 ml RPMI1640, and adding 0.8 ml 2.5% glutaraldehyde (Tousimis, Rockville, Maryland) in Sorensen's buffer (*p*H 7.2, 380 mosmol). This final 1% fixative concentration was selected to correspond to that previously utilised in studies reporting a difference in lymphocyte topography[2-5]. The fixed cells were then washed twice in Sorensen's buffer and processed on poly-L-lysine-coated glass coverslips as described previously[15]. The second method consisted of processing parallel samples by the aspiration filtration method according to Polliack *et al.*[2-5]. From 2×10^6 to 8×10^6 cells suspended in 5.0 ml of media were aspirated live on to silver membrane filters (13 mm diameter; Flotronics, Selas, Spring House, Pennsylvania) in Swinex holders (Millipore) with a peristaltic pump at a flow rate of 1.0 ml min^{-1}. When 0.5 ml of the suspension remained above the filter, 2.0 ml 1% glutaraldehyde in Sorensen's buffer were added dropwise. After 10 min, the filters were transferred to small plastic Petri dishes for continuing fixation in fresh fixative for 24–72 h. Fixation was carried out at 22 °C. All specimens were prepared in triplicate. Subsequently, the fixed cells on coverslips and silver filters were dehydrated through a graded series of ethanol and prepared by the critical-point drying method[20]. Samples were processed entirely in the liquid phase to avoid air drying. The specimens were vacuum coated with gold–palladium on a tilting, rotating stage, examined with an Etec Autoscan microscope at 20 kV and 45° tilt, and photographed using Polaroid 55 film. The surface morphology of cells prepared by each of the two methods was classified into 'relatively smooth', 'villous', and 'ruffled' categories to correspond with previous descriptions of leukocyte surface morphology[2-5]. Percentages of smooth cells are reported in Table 1. *a–c*, Human leukocytes fixed in suspension, attached to poly-L-lysine-coated glass coverslips, and processed for SEM[15]. Cells are spherical and uniformly covered with short microvilli. Several ruffled cells and platelets are present. *d–f*, Parallel samples of populations illustrated in *a–c* prepared for SEM by the aspiration–filtration technique used in the descriptive studies[2-5]. The majority of cells shown have lost their villous surface topography. The field in *d* was selected from a filter with a high cell density to illustrate both villous and smooth forms. Ninety-five per cent of the cells were smooth on filters to which fewer cells (2×10^6) had been applied (Table 1). When cell density exceeds a monocellular distribution, cells not in direct contact with the filter tend to retain their villous structure (*d–f*). Smooth lymphocytes tend to be smaller and located within the crevices of the filter (*d–f*). Note the partially villous cell which seems to have been partly aspirated into a filter crevice (*f*). *a* and *d*, Normal peripheral blood leukocytes; *b* and *e*, cells from patient with CLL, B-cell leukaemia; *c* and *f*, cells from patient with Sezary's syndrome, T-cell leukaemia. ×1,750.

By varying the number of cells applied to the filters, we have been able to vary further the ratio of smooth to villous cells in any given population. On filters where the cell density exceeded a monolayer, the surfaces of cells in direct contact with the substrate tended to be smoother than those of cells retained on the filters by way of attachment to other cells (Fig. 1*d*) Many of the smooth cells seemed to have been aspirated into crevices of the filter and such cells tended to be smaller than the more villous cells which were situated on top of the substrate or of other cells (Fig. 1*d*–*f*).

The present investigation demonstrates that smooth lymphocytes can be artefactually and variably generated from villous cells by the SEM preparative technique used in those studies that initially described B cells as villous and T cells as relatively smooth[2–5] The alteration induced by this technique also seems to be nonspecific since it affects normal mononuclear leukocytes as well as leukaemic lymphocytes of both B and T origin. Thus, using the aspiration filtration method[2–5], we have been unable to reproduce the observation that populations of T cells are predominantly smooth, whereas B cells are villous. A likely explanation for these differences lies in the way the experiments of Polliack *et al.*[2–5] were carried out and the manner in which the results were interpreted.

For example, we have demonstrated that the technique of aspiration filtration[2–5] smooths the surfaces of living cells in direct contact with the filters and aspirates portions of some of the cells into the filter crevices. Thus, it seems that the surface characteristics of the final population are likely to depend on the specific conditions used, such as duration of contact with substrate, the negative pressure applied to the filter, and/or cell density. It is not clear that these important variables were controlled adequately in the previous experiments[2–5]. In addition, control of other important factors, such as the conditions in which cells were handled before aspiration filtration has not been reported by proponents of the T–B surface structure dichotomy[2–5]. Cold temperature, for example, has been shown to smooth the surface architecture of lymphocytes[21,22]. Normal T lymphocytes induced to form E rosettes at 4 °C are relatively smooth[3,23], but are villous when E rosettes are formed at 20 °C (refs 8 and 24). R.B. (unpublished) has demonstrated that lymphocytes from the same E-rosetting T-cell population are villous at room temperature and relatively smooth at 4 °C. When all these variables are controlled we observed no difference between the surface morphology of B and T lymphocytes. Thus it is possible that the initial results, that ratios of T to B cells in normal human peripheral blood seemed to correlate with surface morphology, were secondary to one or any combination of these variables and erroneously attributed to a structure–function relationship. Differences in the handling of various samples without regard to these variables could have led to a perpetration of the error with rosetted cells, cell lines and leukaemic populations.

The investigators correlating ratios of villous to smooth cells with proportions of B and T lymphocytes[2–5,25] determined by surface marker studies also failed to appreciate that there is no *a priori* reason to assume that the sets of surface morphological characteristics (that is, smooth and villous) are coextensive with the sets of functional classes of lymphocytes identified by surface markers (that is, T and B cells). This is particularly true when the populations used for markers may not have been comparable with those used for SEM (see ref. 26 for discussion). The analysis of individual cells fixed in suspension and positively identified by SEM surface markers demonstrated no difference in the surface morphology of surface immunoglobulin-positive and -negative cells[12,13].

A study using Hoffmann modulation contrast light microscopy (40× Nikon achromat objective; NA 0.65) with a maximal theoretical resolution just at or below the limits needed to delineate lymphocyte microvilli, failed to detect surface microvilli on most murine lymphoid cells[25]. When living human lymphocytes from monocyte-depleted Ficoll–Hypaque preparations, however, were processed at room temperature and examined with light microscopy (100 × Zeiss Planachromat objective; NA 1.25), 98% were villous (unpublished). These results are consistent with the report[27] that murine θ-positive and surface immunoglobulin-bearing lymphocytes stained with fluoresceinated ligands are equally villous.

In conclusion, it is not possible to identify reliably classes of lymphocytes *in vitro* exclusively on the basis of their SEM surface topography. Our observations challenge the concept of 'villous B' and 'smooth T' lymphocytes and indicate the necessity for a more critical approach to the preparation of cells of all types for SEM. These results do not exclude the possibility that subpopulations of lymphocytes may demonstrate distinctive differences in surface morphology with higher resolution electron microscopy. Nor do they preclude the possibility that lymphocyte subpopulations might respond differentially to certain microenvironmental manipulations with distinctive changes in surface morphology[28]. Because it has been amply demonstrated that lymphocyte surface morphology is dynamic and exquisitely susceptible to changes in *in vitro* conditions[7,10,24,26–28] it is likely that lymphocytes undergo continual alterations in their shape and form as they mature, divide, circulate, migrate and interact with surfaces and other cells as they function *in vivo*.

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Microbial cells living immobilised on metal hydroxides

THE immobilisation of enzymes by attachment to water-insoluble materials has received considerable attention recently[1], both for academic reasons and for possible industrial applications. A logical extension of this approach, especially where multi-stage enzymic reactions are being considered, is the immobilisation of microorganisms, which are often the source of many enzyme preparations. The advantages of such an approach are immediately obvious. The tedious and time consuming procedures for enzyme extraction and purification are instantly eliminated, cofactors and coenzymes are readily at hand, the cellular enzymes are often organised into the requisite metabolic pathways and problems associated with enzyme instability may also be avoided. Furthermore, the use of immobilised cells would avoid the problem in industrial processes of separating the product from the enzyme. The immobilisation of cells by the standard glutaraldehyde procedure, however, causes their decease, and entrapment in polyacrylamide gel is commonly used instead to achieve immobilisation[2,3]. This method produces a minimum of interaction between the microbial cell and the insoluble matrix, thus maximising the probability of the cell's survival. On the other hand, the availability of the cell to any intended substrate is seriously reduced, since it can reach the cell only by diffusion. An alternative approach of immobilising cells on collagen, by the formation of a stable network of multiple ionic (salt) linkages, hydrogen bonds and Van der Waals' interactions has been successful[4]. We have now pursued an independent approach and report here the successful immobilisation of cells on the hydroxides (hydroxyoxides) of titanium (IV) and zirconium (IV) by a chelation process.

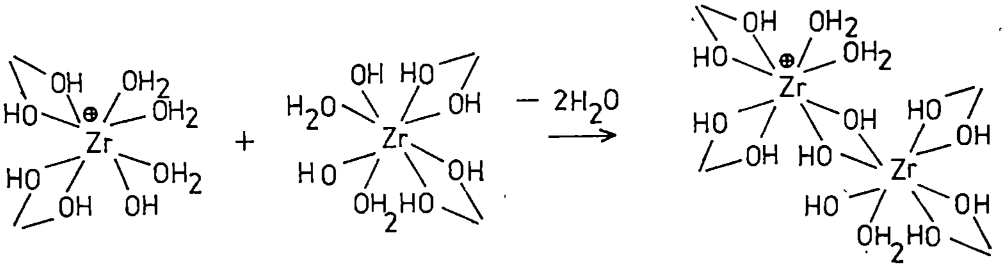


Fig. 1 The polymerisation of hydrolysed zirconium complexes.

Titanium hydroxides are insoluble over the normal physiological pH range, and acting as enzyme immobilisation matrices give good retention of enzymic activity[5,6], they thus seem to have little or no effect on the function of biologically active molecules. If enzymic activity, which is extremely sensitive to conformational changes in the enzyme molecule, is not seriously affected by immobilisation, then there seems to be little reason why cell walls should be disrupted or destroyed by this process and the cells themselves, therefore, have a good chance of remaining viable. Zirconium has an outer electron configuration of $4d^2 5s^2$ and occurs almost exclusively in its compounds in the 4+ oxidation state. Bonding to the maximum possible extent results in a maximum coordination number of 8. The zirconium ion can use various hybrid orbitals to give strongly directional bonds. For eightfold coordination, the orbitals used are d^4sp^3, leading to a dodecahedral arrangement, or d^5p^3, giving a square antiprismatic configuration[7]. In the latter, the lone pairs of electrons in the ligands form coordinate bonds with the vacant 4d and 5p orbitals of the zirconium ion, which are essentially covalent, involving the sharing of the lone pair of the oxygen atom.

The behaviour of zirconium complexes in aqueous solution is characterised by hydrolysis and polymerisation. When zirconium (IV) chloride is dissolved in water the reactions which occur can be formulated as below

$$\mathrm{ZrCl_4 + 8H_2O \rightarrow [Zr(H_2O)_8]^{4+} + 4Cl^-}$$

$$\mathrm{[Zr(H_2O)_8]^{4+} + H_2O \rightarrow [Zr(OH)(H_2O)_7]^{3+} + H_3O^+}$$

$$\mathrm{[Zr(OH)(H_2O)_7]^{3+} + H_2O \rightarrow [Zr(OH)_2(H_2O)_6]^{2+} + H_3O^+}$$

The final complex cation is then able to polymerise to give a tetrameric complex

$$\mathrm{4[Zr(OH)_2(H_2O)_6]^{2+} \rightarrow [Zr_4(OH)_8(H_2O)_{16}]^{8+} + 8H_2O}$$

This tetrameric complex has been shown[8] to be present in the aqueous solution, which has acidic properties, the H^+ activity of a mM solution being almost equal to that of mM HCl (ref. 9). This arises from the hydrolysis of the tetrameric complex

$$\mathrm{[Zr_4(OH)_8(H_2O)_{16}]^{8+} + 4H_2O \rightarrow [Zr_4(OH)_8(OH)_4(H_2O)_{12}]^{4+} + 4H_3O^+}$$

The charge on the complex is thus reduced to 4+ and, depending on the acid concentration, can be reduced further. Each of the four zirconium ions can hydrolyse separately

$$\mathrm{[Zr_4(OH)_8(OH)_4(H_2O)_{12}]^{4+} + H_2O \rightarrow [Zr_4(OH)_8(OH)_5(H_2O)_{11}]^{3+} + H_3O^+}$$

This results in one neutral group in the tetrameric complex. Assuming that the neutral group is localised on one of the zirconium atoms, the other three retaining their single positive charges, then this can be the starting point for a polymerisation reaction. The neutral site reacts with a singly charged site of another tetrameric complex so that the zirconium ions are connected by two hydroxide bridges (Fig. 1). The degree of polymerisation increases as the acid strength decreases[9] and

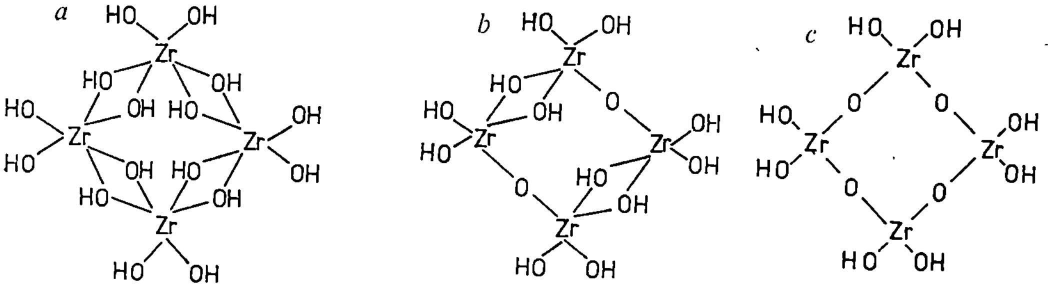
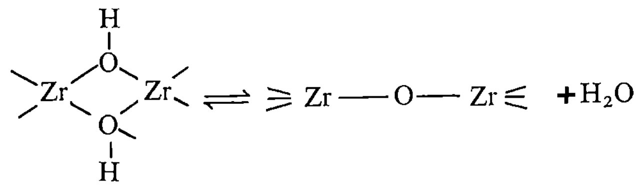


Fig. 2 *a*, *b*, *c*, Structures of zirconium hydroxide.

so addition of alkali to neutralise the acidic solution formed on dissolution of zirconyl chloride also increases the degree of polymerisation. When neutralisation is continued to *p*H 3, a precipitate begins to form. At the equivalence point (*p*H 9) precipitation is complete, the gelatinous precipitate being zirconium hydroxide (also known as hydrated zirconium dioxide or hydrous zirconia).

We propose that the precipitate contains a variety of compounds. The formation of the hydrated oxide is preceded by a stage involving the production of a true hydroxide containing hydroxyl groups[10]. The conversion of the hydroxide into 'hydrous zirconia' takes place very rapidly and irreversibly so that in practice the gelatinous precipitate is not the hydroxide.

Zaitsev[11], however, has shown that freshly precipitated zirconium hydroxide contains four hydroxo-groups for every zirconium atom and therefore that the compound with the formula $Zr(OH)_4.nH_2O$ can exist. Its stability is low, and on ageing it is converted into the so-called zirconyl hydroxide, containing two hydroxo-groups for every zirconium atom: $ZrO(OH)_2.nH_2O$ (ref. 11). When this is dried in the cold to a powder, it is converted into hydrated zirconium dioxide, having the formula $ZrO_2.xH_2O$ and containing no hydroxyl groups. Only two hydroxyl groups per zirconium atom of freshly precipitated zirconium hydroxide react with acid, the other two remaining inactive because double hydroxo- (or olate) bridges are formed between the zirconium atoms. The structure of the freshly precipitated hydroxide is thus extremely likely to be that depicted in Fig. 2*a*, together with an indefinite number of water molecules. This structural formula represents the main unit of the hydroxide, but in certain circumstances some of these tetrameric molecules may be joined together by oxo- or olate bridges. Conversion of a double olate bridge into an oxo-bridge is possible[12] by the following mechanism



$$>\mathrm{Zr}\begin{smallmatrix}\mathrm{O(H)}\\ \mathrm{O(H)}\end{smallmatrix}\mathrm{Zr}< \;\rightleftharpoons\; \geqslant \mathrm{Zr}—\mathrm{O}—\mathrm{Zr}\leqslant \;+\mathrm{H_2O}$$

suggesting that two other zirconium hydroxides may exist having zirconium atoms joined alternatively by double olate and oxo-bridges or zirconium atoms joined only by oxo-bridges. The possible structures of these hydroxides are shown in Fig. 2*b* and *c*; Fig. 2*b* represents a hydroxide having the formula $(OH)_3Zr–O–Zr(OH)_3.n\ H_2O$ and Fig. 2*c* corresponds to the hydroxide with the formula $ZrO(OH)_2.nH_2O$. The infrared absorption spectra of these two compounds indicate that they do not contain the $Zr{=}O$ group, and so must be cyclic polymers of the type indicated. We therefore conclude that in precipitated hydrous zirconium hydroxide there is an abundance of hydroxy groups replaceable by ligands.

Since the immobilisation process for a number of metal hydroxides is envisaged as involving the replacement of hydroxyl groups[13] on the surface of the metal hydroxide by suitable ligands from enzyme or cell, resulting in the formation of partial covalent bonds, we conclude that zirconium hydroxide should be suitable for immobilisation phenomena. In the case of enzymes, such ligands could be the side-chain hydroxyls of L-serine or L-threonine, the carboxyls of L-glutamic acid or L-aspartic acid and the ε-amino group of L-lysine residues, (Fig. 3), oxygen-containing ligands being preferred to those containing nitrogen[14]. In the case of cells, the structural complexity of the cell wall ensures the availability of a great diversity of suitable ligands from both protein and carbohydrate moieties.

Samples (each of 1.3 mmol) of the metal hydroxides for use in cell immobilisation were prepared from solutions of their tetrachlorides (titanium tetrachloride 15% w/v in 15% w/v hydrochloric acid (BDH) and zirconium tetrachloride (BDH) 0.65 M in 1.0 M hydrochloric acid) by the slow addition of 2.0 M ammonium hydroxide to neutrality (*p*H 7.0). The samples were washed with saline solution (0.9% w/v, 3 × 5.0 ml) to remove ammonium ions and then used for cell immobilisation studies as below.

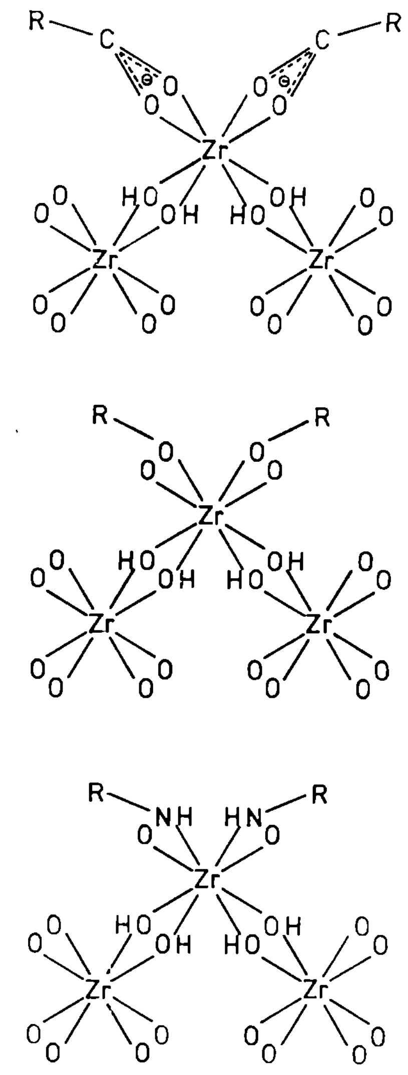


Fig. 3 Projected structures of the complexes of zirconium hydroxide with carboxyl, hydroxyl and amino groups respectively.

The procedure for cell immobilisation is very simple and is illustrated by the following example. A suspension (in 10 ml 0.9% w/v saline) of *Escherichia coli* cells (A_{600} 0.216) was mixed with a sample of the metal hydroxide prepared as above and agitated gently for 5 min at room temperature. The mixture was then allowed to stand at room temperature and the suspension settled out, leaving a clear supernatant (A_{600} 0.022) which was practically devoid of microorganisms (shown by microscopy) The immobilised cell preparation was consolidated by centrifugation at low speed and removed from the supernatant for further examination.

Saccharomyces cerevisiae and *E. coli* cells were immobilised in this manner and the preparations were examined for continued viability by measurement of their oxygen uptake (at 25 °C in aerated 0.2 M sodium acetate buffer *p*H 5.0, or 0.9% w/v saline by use of an oxygen electrode (Pye Unicam Ltd). The rate of oxygen uptake of the immobilised cells was ~30% of that of the same number of free cells. This result showed that respiration of the cells could continue when the cells were immobilised. The reduced rate of oxygen uptake is probably caused by the restriction by the metal hydroxide of access of aerated buffer to the cells and a decrease of the area of cell surface available for oxygen transfer.

To show that the cells were firmly attached to the surface of the metal hydroxide and not just loosely trapped in the gelatinous matrix, a number of arguments are invoked. Free cells of *E. coli* are comparatively small and cannot be centrifuged down to any significant extent in the centrifugation conditions used for collecting the immobilised cells; they are not particularly robust and so any disruption process which had occurred would, therefore, have rendered them inactive and unable to respire. Solutions of bicarbonate, phosphate, fluoride (and so on) ions which have been shown[6] to remove loosely bound proteinaceous and other materials from zirconium (IV) hydroxide were singularly ineffective in the attempted release of the immobilised cells from the matrix.

To show further that the cells were firmly attached to the metal hydroxide, a different microorganism, *Serratia marcescens*, was employed. When incubated in nutrient medium at 25 °C, this organism produces a distinctive red colouration, which enables the immobilised cells to be distinguished readily from those of any other contaminating microorganisms and also obviates the need for sterile experimental conditions.

On adding cultures of *S. marcescens* to zirconium (IV) and titanium (IV) hydroxides, the red colouration (that is, the cells) became associated with the insoluble matrix, the supernatant and subsequent washings (with saline solution) being almost cell free, thus demonstrating the strength of the cell–metal hydroxide interaction. When samples of these immobilised cells were added as a small inoculum to fresh culture medium, growth was observed (as detected by the large increase in numbers of red cells). Since at no time throughout extended studies of the growth of *S. marcescens*, in various conditions of humidity, oxygen tension, temperature and medium composition and ultraviolet irradiation-induced mutation, was release of colour from the cells observed, this was taken as evidence that the cells had suffered no deleterious effects on immobilisation.

It was also found that cells could be immobilised at lower *p*H, titanium (IV) hydroxide being more effective for this purpose, in the range studied (*p*H 2–5). This phenomenon is useful since not all microorganisms exist in a neutral *p*H environment (for example, *Lactobacillus* and *Acetobacter*) and enabled us to produce a small scale immobilised cell reactor. *Acetobacter* cells, immobilised on titanium (IV) hydroxide, were able to produce acetic acid from an alcoholic medium in a continuous reactor, often at rates higher than those obtained when free cells were used (a basic rate 87 g acetic acid per day (86% conversion) increased to 263 g per day (99% conversion) after immobilisation), thus demonstrating the practicability of this immobilisation method. If this simple means of cell immobilisation was applied to other microorganisms it could well result in further immobilised cell reactors of this sort, for the selective production of commercially important biochemical and pharmaceutical compounds. Furthermore, we conclude from the results that immobilised cells can retain their activity for a matter of weeks at least.

As with immobilised enzymes, immobilised cell systems have been applied to very few industrial processes, partially because of the inertia of established methods and partially because of the increased difficulty of operating such systems. It seems certain, however, that the inherent advantages of these systems will eventually prevail and there will be an increasing use of immobilised systems by the pharmaceutical, chemical and food industries.

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Phenotypic difference between *uvrA* and *uvrB* mutants of *E. coli*

THE synthesis of colicin E1, an antibiotic protein, by cells of *Escherichia coli* is coded for by a bacterial plasmid, colicinogenic factor E1 (ColE1). The synthesis is induced by agents such as ultraviolet light[1] or mitomycin C (mit C)[2]. Ultraviolet and mit C are known to modify the base portions of DNA to form pyrimidine dimers[3] and cross linkages between complementary strands[4], respectively. Damage to DNA caused by ultraviolet or mit C is repairable by a similar molecular mechanism[5,6], but the relationship between these repair systems and the induction process of ColE1 synthesis has not been established unequivocally We have analysed the induction of the colicin by ultraviolet and mit C in various ultraviolet-sensitive mutants to which the colicinogenic factor was transferred. Among the mutants tested, the *recA* mutant did not synthesise the colicin in response to ultraviolet or mit C as reported previously[7]. In addition, however, we have now found that the induction of the colicin synthesis is greatly delayed in the *uvrB* mutant, but is normal in the *uvrA* mutant.

Both *uvrA* and *uvrB* mutants are deficient in removing pyrimidine dimers, or cross linkages caused by mit C,

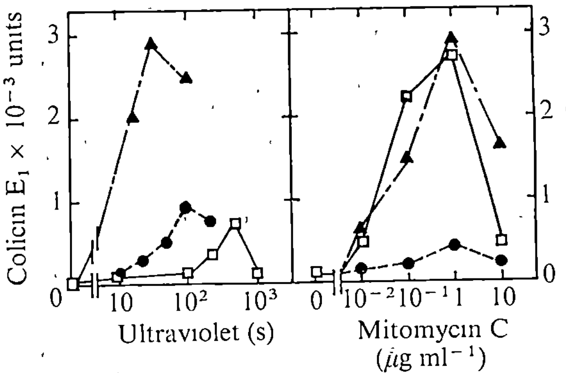

Fig. 1 Effect of varying doses of ultraviolet light and mitomycin C on the induction of colicin E1 synthesis The following strains of *E. coli* K12 were used: AB1157 (uvr^+, thr^-, pro^-, leu^-, his^-, arg^-, thi^-, lac^-, gal^-, ara^-, xyl^-, mtl^-, tsx^r, str^r, F^-), AB2437 ($uvrA^-$, his^-, thi^-, str^r, Hfr), and AB1885 (AB1157 $uvrB^-$) ColE1 DNA was transferred to these strains according to the method previously described[14]. For the growth of bacteria, minimal salt media (M-9) supplemented with vitamin-free casamino acids (0.15 %), thiamine (10 μg ml^{-1}) and glucose (0.4%) were used[14] Cells of each strain which were aerobically grown to log phase (1×10^9 cells ml^{-1}) were washed and incubated without aeration in the above synthetic media at 37 °C in the dark after addition of mit C or irradiation with ultraviolet light After 2 h the incubation mixtures were treated by sonic oscillation and the colicin activities were determined as previously described[14]. The colicin activity of untreated cells (less than 20 units per A_{660}) was subtracted from activities obtained. For ultraviolet induction, the bacterial suspension (2.5 ml) was irradiated in 9-cm diameter Petri dishes with a 6 W low pressure mercury National germicidal lamp from the height of 1 m. In these conditions, irradiation for 60 s is equivalent to 30 erg mm^{-2}. □, uvr^+; ▲, $uvrA^-$; ●, $uvrB^-$.

Table 1 Time course of the induction of colicin E1 synthesis by mitomycin C and decarbamoyl mitomycin C

Strain	mit C-induced colicin E1 activity at (h)					DCmit C-induced colicin E1 activity at (h)	
	0	1	2	4	8	0	2
AB1157 (ColE1) *uvr*$^+$	0	25	100	69	54	0	90
AB2437 (ColE1) *uvrA*$^-$	0	50	100	42	26	0	100
AB1885 (ColE1) *uvrB*$^-$	0	5	10	33	80	0	81
KMBL49 (ColE1) *uvr*$^+$	0	56	100	100	100	0	88
KMBL90 (ColE1) *uvrB*$^-$	0	1	20	38	53	0	70
KMBL101 (ColE1) *uvrB*$^-$	0	14	32	32	70	0	68

AB1157 (ColE1), AB2437(ColE1), and AB1885(ColE1) were described. KMBL90 *dar*$_1$$^-$ (*uvrB*$^-$) and KMBL101 *dar*$_6$$^-$ (*uvrB*$^-$) were derived from KMBL49 (KA22 *dar*$^+$, *thr*$^-$, *leu*$^-$, *thi*$^-$, *pyr*$^-$, *thy*$^-$)[11]. For the growth of these KMBL strains, M-9-glucose-casamino acids media (see Fig. 1) were further supplemented with thymine (20 μg ml^{-1}), uridine (200 μg ml^{-1}), and biotin (4 μg ml^{-1}). Transfer of ColE1 DNA to these strains was made from *nadC*13(ColE1) with the aid of W3110 prototroph F$^+$. The concentration of mitomycin C or decarbamoyl mitomycin C was 1 μg ml^{-1}. Conditions for induction and determination of colicin E1 activity have been described previously.

compared with the *uvr*$^+$ strain[5,6,8] and are considered to be defective in the endonucleolytic steps of excision repair[9]. Apart from the difference at the genetic locus[10,11], no phenotypic differences between these two mutants have been found with respect to the following points at least: sensitivity to ultraviolet, mit C (ref. 5) and X rays[10]; the capacity of ultraviolet or mit C to induce mutations[6,12]; the ability to reactivate ultraviolet-irradiated T1, or λ phage[5,13]; the inducibility of prophage by ultraviolet or mit C (ref. 13); and the absence of endonuclease activity[9]. As far as colicin E1 induction was concerned, however, the *uvrB* mutant behaved differently from the *uvrA* mutant and this provides evidence for the first time that the functions of *uvrA* and *uvrB* genes are not completely identical.

Cells of the *uvrA* and *uvrB* mutants and a *uvr*$^+$ strain[10] which were made colicinogenic were treated with various doses of ultraviolet and mit C and incubated for 2 h in the standard induction conditions[14]. In the case of ultraviolet induction, both mutants synthesised greater amounts of colicin E1 in response to lower doses of ultraviolet than did the wild type (Fig. 1). In contrast, on mit C induction, little colicin E1 was produced in *uvrB* mutants at any concentration of mit C, whereas colicin E1 synthesis in *uvrA* mutants was almost the same as in the *uvr*$^+$ strain (Fig. 1). The optimal concentrations of mit C for induction were the same for these mutant strains, whereas the optimal doses of ultraviolet for induction were different in the two strains. Ranking the mutants according to the ultraviolet dose required for maximum colicin induction correlated with the order of sensitivity to ultraviolet-induced killing; the *uvrA* mutant was the most sensitive to ultraviolet and the optimal dose for colicin induction was also the lowest.

In the *uvrB* mutant, however, when the cells were incubated for a longer period, the colicin was gradually synthesised (Table 1). After 8 h, the mutant produced almost the same amount of colicin E1 as the *uvr*$^+$ strain. The same findings were obtained from other *uvrB* mutants such as KMBL90(ColE1) and KMBL101(ColE1)[11]. Thus, it can be concluded that colicin E1 synthesis was greatly delayed in *uvrB* mutant.

Viable cell number of the *uvrB* mutant decreased to 1% after a 30-min incubation with mit C (1 μg ml^{-1}). In addition, when the *uvrB* cells were incubated for 30 min with mit C, followed by washing and additional incubation in the fresh medium without mit C, the same time course of colicin E1 synthesis was obtained as without treatment. These observations suggest that the first step of the action of mit C is already complete within 30 min of incubation in the *uvrB* mutant and that a certain process after this step to colicin E1 protein synthesis is impaired.

Decarbamoyl mitomycin C (DCmit C), a monofunctional alkylating agent, could induce colicin E1 synthesis at a normal rate in all of the above *uvrB* mutants (Table 1). DCmit C attaches to a single strand of DNA and does not cross link between the strands[6]. In addition, the DNA damage caused by the monofunctional mit C was repairable by an excision repair system similar to that for ultraviolet-irradiated DNA. Thus, the delayed response of the colicin synthesis in *uvrB* mutant was associated with the formation of the cross linkages between the DNA strands.

Although the *uvrA* mutant lacks the ability to repair DNA damage caused by mit C, DCmit C and ultraviolet[5,6] by excision, the mutant was induced to synthesise colicin E1 by these agents. It is, therefore, unlikely that the excision repair process itself is directly involved in colicin induction. In these mutant strains, another repair mechanism, the post-replicational repair system, will operate in some cases[15]. The operation of post-replicational repair seems to be essential for colicin induction, since neither mit C nor ultraviolet could induce colicin E1 synthesis in a *recA* mutant. The *recA* gene is believed to control the whole process of the post-replicational repair system[15].

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An intracistronic region of gene *A* of bacteriophage φX174 not involved in progeny RF DNA synthesis

GENE *A* of the single-stranded DNA bacteriophage φX174 has a key position in the replication of the first double-stranded replicative form (parental RF) to progeny RF DNA molecules. The action of gene *A* manifests itself in the introduction of a nick in the viral strand of the parental RF (ref. 1), which seems to be a condition for the RF DNA replication. The nick is located within gene *A* (ref. 2)

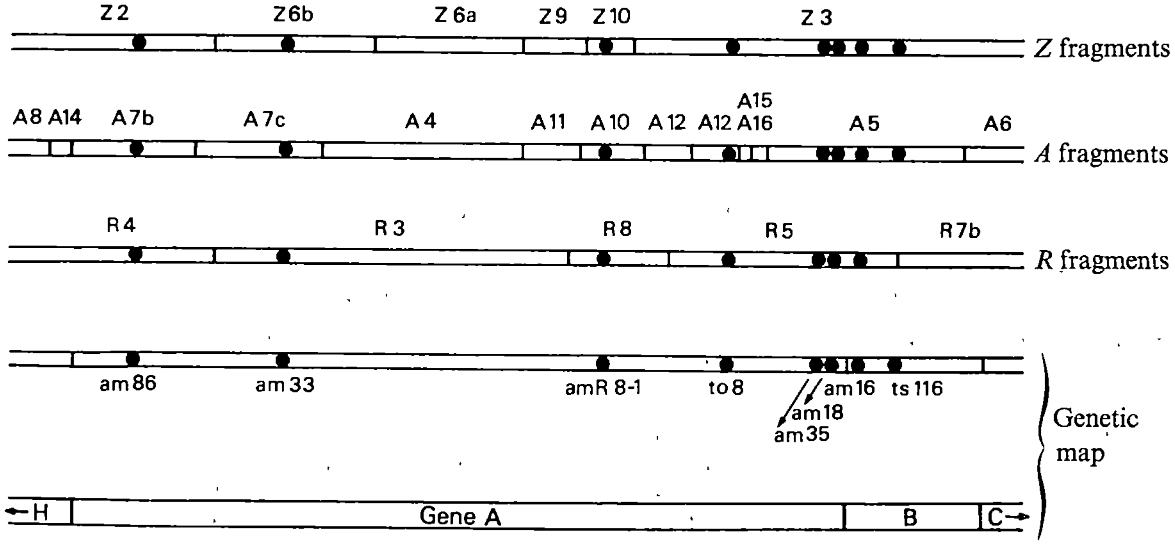

Fig. 1 Genetic map of the gene *A* and gene *B* region.

and is achieved by a *cis*-acting gene product[3,4]. It has been shown that gene *A* codes for two overlapping proteins, *A* and *A′* (molecular weights 35,000 and 60–65,000, respectively)[5,6]. The molecular weight of the large protein corresponds to the size of gene *A* as determined using restriction enzyme fragments[7]. The large A protein may be involved in achieving the nick[8], the small A protein in the shut-off of the host DNA replication[9]. We present here genetic evidence that gene *A* consists of three different regions. Two of them are involved in double-stranded DNA replication. They are physically separated by a third region, characterised by mutations that leave double-stranded DNA synthesis unaffected.

We have isolated a group of conditional lethal nonsense mutants of φX174 which could not be assigned unambiguously to one of the known genes. In complementation tests these mutants complemented well with mutants of genes *A*, *C* up to and including *H*, but the complementation behaviour with mutants of gene *B* was aberrant. This is shown in Table 1 for *to8*, one of the mutants[10] of that group. No complementation could be found between *to8* and the amber mutants of gene *B*, whereas complementation with the gene *B ts* mutants did occur, although marginally (the temperature seemed to be very critical to the selfing of these *ts* mutants).

In the first instance we supposed that *to8* belonged to a new cistron, located (in terms of translation) distally of and adjacent to gene *B* (ref. 12). If so, the complementation behaviour could be explained by a polarity effect of the amber mutants of gene *B* over the mutants of the new cistron. After the development of the new technique of localisation of mutants with the help of restriction enzyme fragments[7], however, we found (Table 2) that the *to8* mutation is covered by the R5 fragment made by the restriction enzyme *Hin*dII from *Haemophilus influenzae* Rd, the Z3 fragment made by *Hae*III from *H. aegyptius*, one of the four A12 fragments made by *Alu*I from *Arthrobacter luteus*, and the R5A12 fragment (= the A12

Table 1 Complementation of *to8* with mutants of all the known φX174 genes

Complementation of *to8* with			Burst sizes			Complementation behaviour
Test mutant	Gene	Temperature (°C)	Mixed infection	Selfing *to8*	Selfing test mutant	
am86	*A*	30	42	0.32	0.21	+
am33	*A*	30	45	0.32	0.26	+
am35	*A*	30	34	0.32	0.24	+
am18	*A*	30	26	0.32	0.26	+
am16	*B*	30	0.28	0.32	0.22	—
amH210	*B*	30	0.22	0.32	0.06	—
ts116	*B*	39	0.84	0.05	0.31	—
		38	4.2	0.37	0.51	?
		37	9.4	0.31	2.4	?
ts6	*B*	39	2.1	0.06	0.05	+
		38	11	0.37	0.06	+
		37	18	0.31	2.8	?
och6	*C*	37	58	0.05	0.76	+
am42	*D*	39	23	0.01	0.01	+
am3	*E*	39	64	0.03	0.10	+
ts41D	*F*	41	1.9	0.02	0.02	+
am9	*G*	41	18	0.01	0.01	+
ts4	*H*	39	53	0.02	0.35	+

Escherichia coli C (su^-) cells were grown with aeration to log phase, centrifuged and resuspended in fresh medium to a concentration of 10^8–5×10^8 cells ml^{-1}. Ten minutes after addition of KCN (final concentration 0.003 M) the phage mutants were added at a multiplicity of three of each in the mixed infection and of six in the selfings. After a 10-min adsorption period the unadsorbed phage were inactivated by incubation with φX174 antiserum for 10 min. The adsorption mixture was diluted 10^4 times in prewarmed medium and after a 1-h growth period the yield of mutant particles was determined by plating on *E. coli* HF4712 (su^+) at 30 °C. In the combination with *och6* the growth period was shortened to about 30 min, and the yield of the mixed infection and of the selfings was scored on *E. coli* WWU (*su*VIII) at 30 °C. Growth medium for *E. coli* WWU was TPG medium[11] supplemented with 0.05 g methionine, 0.05 g tryptophan, 0.05 g proline, 0.04 g thymidine and 0.03 g cytidine l^{-1}. Other growth media were as described previously[10]. Each combination of mutants was tested in at least two different experiments. Test mutants were provided by Dr R. L. Sinsheimer *et al.*, except for *amH210* which was provided by Dr M. Hayashi.

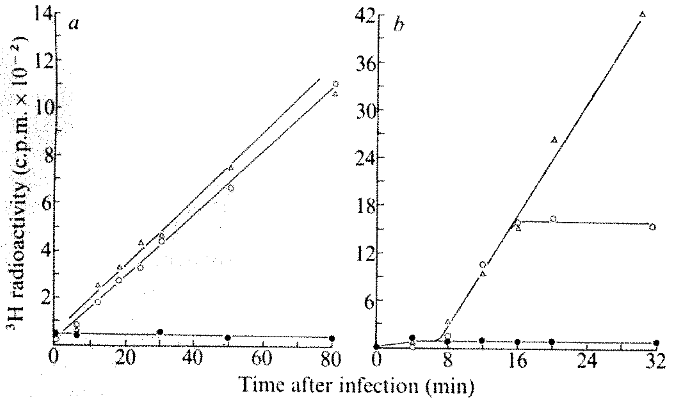

Fig. 2 Measurement of DNA synthesis. *E. coli* HF4704 (thy$^-$, *uvrA*, *su*$^-$) was grown in TPG medium[11] containing 200 μg casamino acids and 2 μg thymine ml^{-1} to a concentration of 3×10^8 cells ml^{-1} and treated with mitomycin C (50 μg ml^{-1}) for 25 min at 37 °C in the dark to block host DNA synthesis. The cells were centrifuged down and resuspended in fresh TPG medium containing 200 μg casamino acids and 1 μg thymine ml^{-1}, plus 35 μg chloramphenicol (CAM) ml^{-1} (*a*) or without CAM (*b*). After a 10-min incubation period at 37 °C ^{3}H-thymidine (specific activity 40 μCi μg^{-1}) was added to a final concentration of 2.5 μCi ml^{-1}, simultaneously with the phage (multiplicity of infection = 3). DNA synthesis was measured by the incorporation of ^{3}H-thymidine into TCA-precipitable material. The precipitation was accomplished by adding 100 μg bovine serum albumin as a carrier to a 0.5-ml sample. After 20 min in the cold the precipitate was spun down and washed twice with cold TCA, solubilised in 1 ml 0.55 N KOH and counted for radioactivity in a liquid scintillation counter. A correction was made for non-infected cells. ○, *to8*; ●, *am33*; △, *am3*.

The observation that *to8* is not affected in the progeny RF DNA synthesis, although it maps within gene *A*, leads to a division of gene *A* into three functionally distinct regions. Two regions are involved in the progeny RF DNA replication. They are separated by a third region in which mutations permit normal progeny RF DNA synthesis. Also, these mutants do not show the asymmetry in complementation as mutants in both the other regions do[10].

How can we interpret these results in terms of the functioning of gene *A*, considering our present knowledge? Both *am86* (*A*) and *am18* (*A*) interfere with the synthesis of the 60,000 molecular weight protein[9], although they are separated by the *to8* region that seems to have a different function. The complementation results indicate three separate genes, two of which are involved in the progeny RF DNA synthesis in such a way that mutants in both functions do not complement each other. The 60,000 molecular weight protein could then be a read-through product, without any function. This, however, is in conflict with the endonuclease activity ascribed to this protein[8].

On the basis of our experiments we prefer the hypothesis of three separate cistrons. Probably the real situation is even more complex, considering the finding that *to8* can be complemented by the *ts* mutants of gene *B*, but not by its amber mutants. This suggests a functional relationship between the products of the genes *A* (*to8* cistron) and *B*. Work is in progress to elucidate these phenomena.

We tried to estimate the size of the *to8* region by inducing amber mutations in that part of gene *A* by the technique of fragment mutagenisation[18]. We found three mutants which behaved like *to8* in the complementation test, and all mapped on the R5A12 fragment. Several 'real' gene *A* mutants were isolated, of which *amR8-1*, located at the R8 fragment, is nearest to *to8* (Fig. 1). So the maximum size of the *to8* region is the maximum distance between *amR8-1* and *am35* (ref. 7), which is about 600 base pairs.

Table 2 Localisation of *to8* mutation with restriction enzyme fragments

R fragment	Ratio wild type–total	*Z* fragment	Ratio wild type–total	*A* fragment	Ratio wild type–total	*R5* digested with *Alu*I	Ratio wild type–total
R3	7.8×10^{-5}						
R8	1.7×10^{-5}	*Z10*	4.5×10^{-5}				
R5	1.2×10^{-2}	*Z3*	1.5×10^{-2}	*A12**	1.2×10^{-3}	*R5A12*	6.7×10^{-3}
*R7**	2.8×10^{-5}	*Z7*	2.9×10^{-5}	*A5*	1.0×10^{-5}	*R5A5*	1.6×10^{-5}
*R6**	1.0×10^{-4}			*A6*	2.6×10^{-5}		
R1	1.3×10^{-4}						
R9	4.6×10^{-5}						
R10	2.1×10^{-5}						
R2	6.9×10^{-5}						
R4	4.0×10^{-4}						

Wild-type double-stranded DNA molecules were completely digested with *Hin*dII (giving rise to *R* fragments, *Alu*I (*A* fragments) and *Hae*III (*Z* fragments). Subsequently the fragments were fractionated with gel electrophoresis[13,14]. *R5* fragment was completely digested further with the *Alu*I enzyme. Subfragments *R5A5* and *R5A12* were used in the experiments. The fractionated double-stranded DNA fragments were annealed to *to8* single-stranded DNA[7]. These partial hybrid molecules were given to spheroplasts of *E. coli* K58 (*su*$^+$) and after a 2–3-h growth period the yield was determined on *E. coli* HF4712 (total yield) and on *E. coli* C (wild type). The rationale behind the experiment is that if the *to8* mutation is covered by a fragment carrying the wild-type allele, the share of wild-type particles in the yield after spheroplast infection (that is, the ratio wild type–total) will be increased markedly[7,15]. The fragments are ordered according to their sequence on the genetic map. The localisation with the *R* fragments showed *R5* as the fragment with the wild-type allele. So, of the *Z* and *A* fragments only the ones in the region of *R5* had to be tested.

*The *R6*, *R7* and *A12* preparations contain respectively 3, 2 and 4 different fragments of the same size.

fragment made from R5 by *Alu*I digestion). In this way we were able to insert *to8* in the genetic map (Fig. 1). The other mutants of this 'new cistron' also map on the R5A12 fragment. It is clear that *to8* maps within gene *A*, and is separated from gene *B* by the *A* mutants *am35* and *am18*.

We next looked at the DNA synthesis in restrictive conditions in the presence and absence of chloramphenicol (CAM). With CAM only progeny RF DNA synthesis is permitted[16]; without CAM both RF and single-stranded DNA synthesis can proceed. As a control *am3* (gene *E*) and *am33* (gene *A*) were included in the experiment. Figure 2*a* shows that *to8* synthesises progeny RF DNA at the same rate as *am3*, a lysis-defective mutant not blocked in DNA replication[16]. Without CAM (Fig. 2*b*) the DNA synthesis of *to8* stops at a time when single-stranded DNA synthesis starts[17]. The gene *A* mutant *am33* shows no significant DNA synthesis in both experiments.

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Expression of flagellar genes carried by bacteriophage lambda

THE specific interaction of many components is necessary for the assembly and function of flagella in *Escherichia coli*. Twenty genes have been shown to participate[1–3] and at least eleven polypeptides are associated with the structure of the flagellar organelle[4]. The synthesis and assembly of these components and of flagellin, the major flagellar subunit protein, is tightly coupled and regulated[5–7]. It has not been possible to analyse these regulatory mechanisms in great detail because: (1) the tight coupling between events usually leads either to the synthesis of a complete organelle, or to no detectable flagellar structure, and (2) most of the flagellar components are present at low levels and their synthesis is difficult to measure. To analyse the hierarchy of control and regulation in this system we have prepared a series of hybrid transducing phages that carry different combinations of flagellar genes. We describe here one of these phages and show that it can be used to study the expression of flagellar gene activities.

Hybrid lambda (λ) *E. coli* DNA molecules were constructed using λgt–λc DNA as the vehicle and isolated F factors as a source of DNA carrying flagellar genes. Figure 1 shows the results of *Eco*RI endonuclease digestion of two of these episomes. F 1338 carries about 2% of the *E. coli* chromosome (Fig. 1) and includes most of the genes that are involved with flagellar assembly and function. F 1906 is a deleted episome[8] derived from F 1338; it lacks more than half the flagellar genes. Approximately 20 distinct bands can be distinguished in the digest of F 1906, and 33 bands in the digest of F 1338. The F 1338 digest was used to prepare hybrids according to the procedures described by Thomas *et al.*[9]. After transfection with hybrid molecules, a large number of λ plaques were picked and purified and lysates were prepared. The lysates were tested for their ability to transduce specific flagellar genes by using them to cross streak various λ lysogens on motility plates. Each lysogen carried a different flagellar gene mutation. Phages that carried specific flagellar genes were able to complement the appropriate lysogen which could then form trails in motility agar. Both abortive transduction and recombination could be scored on the motility plates. In this way various transducing phages have been isolated. We will describe one of these λ*fla*1.

Tests for abortive transduction showed that λ*fla*1 carried the genes *hag*, *fla*N and *fla*B. The *hag* gene controls the structure of flagellin, the major subunit component of the flagellar filament. *Fla*N and *fla*B refer to genes that include mutations resulting in bacterial strains that have no recognisable flagellar filaments. *Eco*RI endonuclease digestion and Agarose gel electrophoresis of λ*fla*1 DNA gave three bands. The relative mobility of the upper band suggested that it represented the left-hand fragment of λgt–λc. The second band had a mobility which corresponded to the right-hand fragment of λ (1.1×10^7 daltons). The third band is barely resolved from the others; it corresponds to the incorporated piece of *E. coli* DNA. Its relative mobility suggests that it is one of the high molecular weight bands seen in the digest of F 1338, but not in F 1906.

To characterise further the incorporated DNA, a series of deletions of λ*fla*1 was prepared. Analysis by abortive transduction revealed the genetic functions that were deleted from each phage, and the Agarose gel electrophoresis patterns of *Eco*RI endonuclease digests showed the corresponding portion of DNA that was removed (Fig. 2). Deletions extending into the incorporated piece of DNA from the right-hand side can remove the *Eco*RI site and fuse the incorporated piece to the right-hand fragment, thus decreasing its mobility on electrophoresis. The same kind of argument applies to deletions entering from the left-hand side and from the middle of the incorporated DNA. The subsequent changes in the apparent molecular weight of the fragments facilitate determination of the relative orientation of the genes with respect to the two ends of λ (Fig. 2).

The deleted phages were used to study the expression of *E. coli* gene activity by measuring incorporation of amino acids into proteins synthesised in ultraviolet-irradiated λ lysogens. Essentially the same procedure was used as that

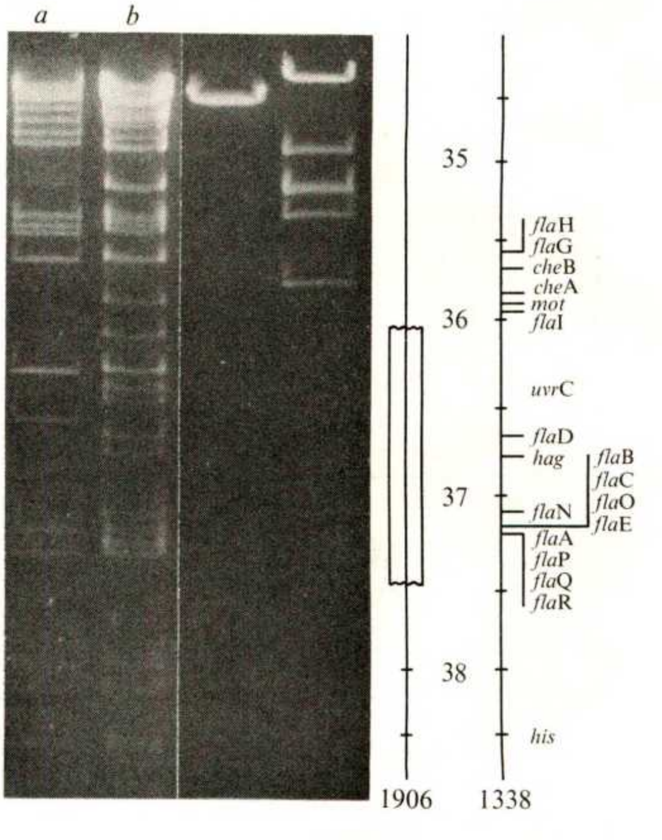

Fig. 1 Electrophoresis of *Eco*RI endonuclease digests of episomal DNA. The episomes were isolated by Sarkosyl lysis[14]. After an initial caesium chloride–ethidium bromide equilibrium centrifugation, the major portion of the chromosomal DNA was withdrawn without perturbing the band of episomal DNA. The solutions containing the episomal DNA were pooled and recentrifuged. Finally, the band corresponding to the density of covalently closed circular DNA was removed carefully. The DNA was precipitated[15] and 2–4 μg was treated with an excess of *Eco*RI endonuclease. After incubation with the enzyme, the samples were heated at 60 °C for 5 min and placed on 0.8% Agarose gel for electrophoresis. On the left is the pattern obtained from F 1906 DNA (*a*) and next to it the electrophoretogram of the digest of F 1338 DNA (*b*). For comparison, the *Eco*RI-treated plasmid (*c*) PML1 (9×10^6 daltons)[16] and λ (*d*) DNA are shown. The map on the right shows the genetic loci carried by the episomes.

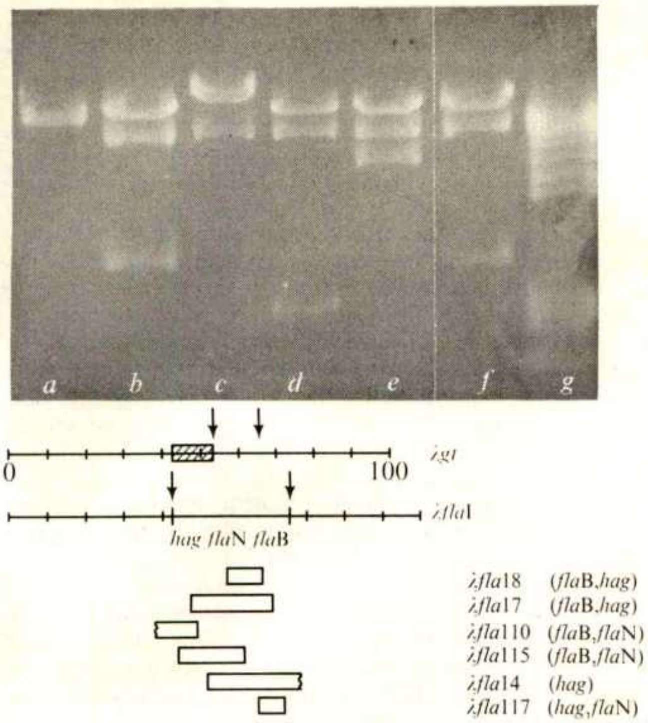

Fig. 2 Mapping of deletions in λ*fla*1. The Agarose gel electrophoretogram shows the results of *Eco*R1 digestion of DNA obtained from deletion mutants of λ*fla*1. The deletions were selected by the pyrophosphate shock technique[17]. Surviving plaques were picked and lysates prepared. After genetic tests to determine the remaining markers, DNA was prepared from the phages by phenol extraction. The DNA was treated with *Eco*RI endonuclease and electrophoresis was on 0.8% Agarose gels. *a*, λ*fla*14; *b*, λ*fla*115; *c*, λ*fla*110; *d*, λ*fla*17; *e*, λ*fla*18; *f*, λgt–λc; *g*, F 1906. The map below is derived from the data in this and other electrophoretograms. The order of the *E. coli* genes is shown. The relative distribution corresponds to that seen on the map in Fig. 1: *fla*N is close to *fla*B and further away from *hag*.

described by Jaskunas *et al.*[10-12]. Figure 3 shows that a major band consistently appears in the acrylamide gel electrophoresis patterns of extracts of cells infected with λs carrying the *hag* gene. Phages that do not carry *hag* but carry either the *fla*B or the *fla*N gene, or both, do not show this band. The band has a mobility which corresponds to an apparent molecular weight of 54,000, and it seems to have two minor bands associated with it, one at about 56,000 and another at 60,000. Furthermore, as the extract is heated at 100 °C for 20 min almost all the material shows a mobility corresponding to a molecular weight of 60,000. This pattern is characteristic of *E. coli* flagellin[13]. The evidence that this band corresponds to flagellin can be summarised as follows. (1) It comigrates precisely with authentic flagellin on one-dimensional acrylamide gels and shows the same characteristic band pattern. (2) It comigrates precisely on two-dimensional acrylamide gels prepared by the techniques described by O'Farrell[14], with authentic *E. coli* flagellin. (3) It only appears in extracts of cells infected with phages that have been shown by genetic techniques to carry the *hag* gene, which is the structural gene for flagellin. (4) Material forming the band which corresponds to flagellin is precipitated by anti-flagellar antibody and removed from the cells by shearing.

To test the regulation of flagellin synthesis, several mutant strains carrying flagellar gene mutations were prepared. Figure 3 shows the results obtained from infections of *hag*, *mot* and *fla*I mutants by λ*fla*1. Clearly flagellin is synthesised in the host strains which carry *hag* (Fig. 3*f* and *i*) or *mot* (Fig. 3*h*) mutations but it is not synthesised in the host which carries the *fla*I (Fig. 3*g*) mutation. Earlier experiments with flagellar mutants[7] suggested that the *fla*I gene product is necessary for *hag* gene expression. This system may be useful for studying the interactions between the *fla*I gene product and the *hag* gene.

We have not so far defined bands on acrylamide gels which correspond to products of the *fla*B or *fla*N genes. If these genes specify structural components of the flagellar basal structure, they may be read at rates that are 100 to 1,000 times slower than flagellin; this could account for our not having observed them. It should be possible, however, to prepare phages where these genes are oriented so that their expression can be affected by the leftward λ promoter. Jaskunas *et al.*[12] have shown that such an arrangement leads to synthesis of *E. coli* gene products at relative rates determined by the λ promoter.

The hybrid phages seem to make available a new way of looking at the regulation of synthesis, assembly and function of the bacterial flagellar organelle.

We thank Jeff Velten and John Abelson for help, Vickers

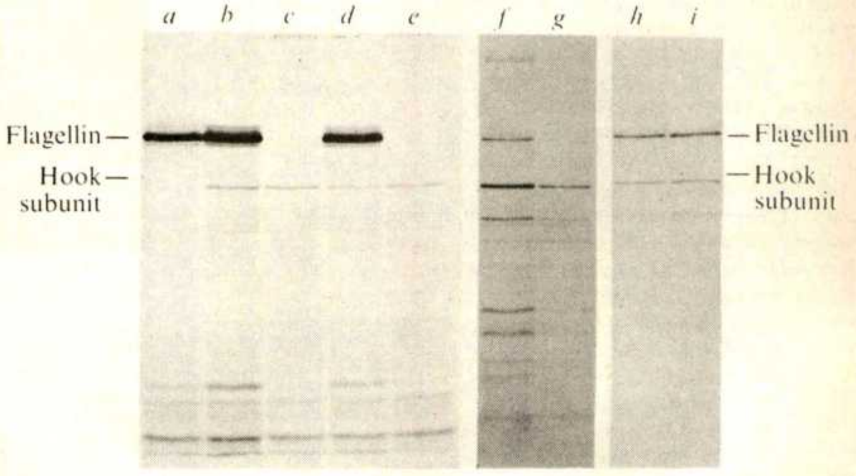

Fig. 3 Electrophoresis of proteins synthesised in ultraviolet-irradiated bacteria infected with hybrid λ phage. The infection of ultraviolet-irradiated cells was performed exactly as described by Jaskunas *et al.*[10,11]. After the infected, irradiated cells were labelled, they were pelleted and resuspended in sodium dodecyl sulphate (SDS)–gel sample buffer (3% SDS, 5% 2-mercaptoethanol, 10% glycerol and 0.06 M Tris–HCl, *p*H 6.8), heated for 1 min at 100 °C, and electrophoresed. Radioactively labelled marker proteins were used and the samples were loaded on to a 12.5% polyacrylamide gel slab containing 0.1% SDS. After electrophoresis the gels were dried and subjected to autoradiography. The host strain was either *E. coli* K12 strain 159 carrying λ (obtained from Dr H. Murialdo)[19], or mutants derived from this strain by mutagenesis and selection with bacteriophage chi. The chi-resistant mutants derived from 159 λ were screened by complementation and strains carrying *hag* (MS6101), *mot* (6106) and *fla*I (6103) mutations were chosen. Samples *a*, *b*, *c*, *d*, and *e* show the results of infection of 159 λ with: *a*, λ*fla*14 (*hag*); *b*, λ*fla*18 (*fla*B, *hag*); *c*, λ*fla*115 (*fla*B, *fla*N); *d*, λ*fla*117 (*hag*, *fla*N); *e*, λ*fla*110 (*fla*B, *fla*N). These autoradiograms were incubated with film for 5 d. The strains carrying flagellar mutations were infected with λ*fla*I at a multiplicity of approximately 10. *f*, *hag* (MS6101), a clear band corresponding to flagellin is seen. *g*, *fla*I (MS6103), no apparent flagellin material was found. *h*, *mot* (MS6106), a flagellin band appeared. *i*, *hag* (MS6101) was infected with λ*fla*14—another λ phage carrying the flagellin gene at a multiplicity of approximately 10. These autoradiograms were incubated with film for 1 d.

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"Non-rigid" nucleotides in tRNA: a new correlation in the conformation of a ribose

WE have reported the three-dimensional structure of yeast tRNA^{Phe}, determined by fitting a skeletal wire model to an electron density map at a resolution of 2.5 Å, phased by the method of isomorphous replacement[1]. Atomic coordinates have been published for the unrefined structure[2]. We are in the process of refining our structure using difference Fourier techniques together with the real-space refinement procedure of Diamond[3]. The crystallographic residual (R) at 2.5 Å is 0.31, a value comparable to that found for proteins at a similar stage.

On the basis of a survey of the known nucleotide and nucleoside structures, several groups have suggested that the conformational angles are naturally confined to a few narrow ranges[4,5], and this concept has been embodied in the term "rigid nucleotide"[5]. The essence of this idea is that although a single nucleotide unit in a polymer or oligomer has six main-chain dihedral angles, the two on either side of the phosphorus atom (ω and ω') are the only ones which are free to vary, the others being confined to a narrow range. By applying this concept to folded nucleic acids, attempts have been made to predict the structures of non-helical regions[5,6]. Refinement of the tRNA structure tests to what extent these ideas and predictions are correct. We have found that the polynucleotide chain is able to explore many more conformations than are implied by the rigid nucleotide hypothesis, and thus that most previous energy calculations (for example ref. 7) and structural speculations have too limited a basis.

While we were building the wire model to fit the isomorphous replacement density map, it became clear that some ribose rings had to adopt the C2′-*endo* conformation (in contrast to the more usual C3′-*endo* found in RNA double helices), to fit the density for the pendant base and the phosphate groups on either side. This change in the sugar pucker implies a change of the dihedral angle ψ' (Fig. 1) from 83° to 146°, and thus has a considerable effect on the course of the chain. Most of the loops and extended regions in tRNA involve several such unusual sugars.

As well as changing the sugar pucker, we were obliged, in fitting the map, to change the conformation at the C4′–C5′ bond in a number of places. The normal conformation here (Fig. 1*a*) is the so-called *gauche*$^+$ (g^+, also called *gg*) in which the angle ψ (O5′–C5′–C4′–C3′) is +60°. (The sign convention is that, looking along the C5′–C4′ bond, a rotation of the nearest bond clockwise by 60° leads to an eclipsed conformation.) In addition to a number of residues in which conformation about the C5′–C4′ bond, is *trans* (*t* or *gt*), with $\psi=180°$, we found four instances in which the value of ψ is $-60°$ (the rare g^-, or *tg*, conformation). In all four of these cases, the ribose ring has the C2′-*endo* pucker. The reason for this correlation is clear: in a C3′-*endo* ribose with $\psi=-60°$ (g^-), the distance between O3′ and O5′ would be only 3.04 Å. By changing the ring pucker to C2′-*endo*, this unfavourable electrostatic interaction is relieved, the O3′–O5′ distance increasing to 3.96 Å (Fig. 2).

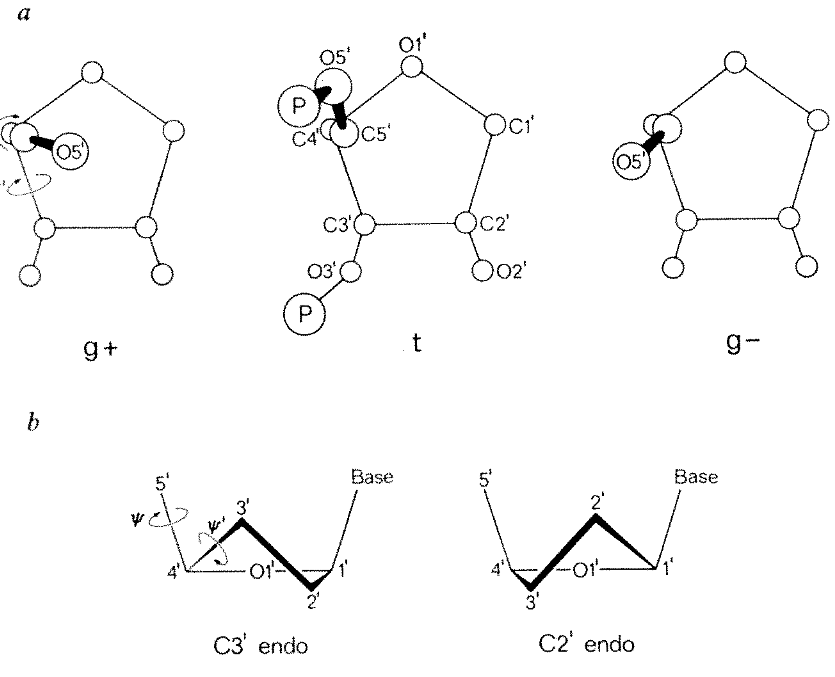

Fig. 1 Schematic diagrams of the ribose ring showing the approximate conformations at (*a*) the C4′–C5′ bond, torsion angle ψ, and (*b*) the C3′–C4′ bond, torsion angle ψ'. In *a* the ribose is viewed face on, and in *b* edge on with the pucker exaggerated.

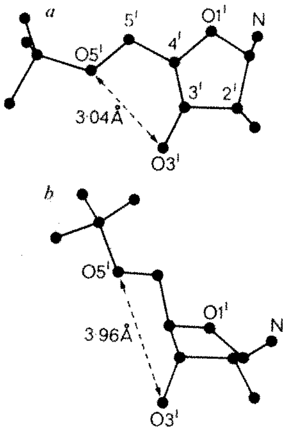

Fig. 2 Projection of a nucleotide on to the plane O3'–C3'–C2' in the two conformations g^-, C3'-*endo* (*a*) and g^-, C2'-*endo* (*b*). Most of the base has been omitted for clarity.

The *t* conformation at C5'–C4' is quite common in nucleosides and 3'-nucleotides and may occur in "kinked" DNA double helices[8]. In tRNA, we believe it occurs at the riboses on either side of the guanine residue involved in the G–U "wobble" base pair[1]. The g^- conformation, however, is much rarer, having been found in only seven nucleoside crystal structures[5,9]. On inspection of these structures, we find, in agreement with our results on tRNA, that in all seven, in which nine crystallographically independent molecules are represented, all the ribose rings have either the C2'-*endo* or the closely related C1'-*exo* conformation. In one of the cases (5'-bromo-5'-dA)[10], the O5' atom is replaced by bromine; in all of the others for which full details are available, the O5' can form intermolecular hydrogen bonds, and this presumably provides the driving force towards the unusual structure. Although no known nucleotides have the g^- conformation, the further interactions occurring in polynucleotides (for example base-stacking and intercalation) may sometimes favour the unusual staggered forms.

It is clear that loop formation in folded nucleic acids is not as straightforward as hitherto supposed; although all the ω–ω' pairs in our refined tRNA structure fall in energetically favourable regions of the ω–ω' plot[7], we must take into account the two extra factors of sugar pucker and rotation about C5'–C4' when considering loop formation. The anticodon loop is the only example in which the folding is achieved by varying only ω and ω'.

Coordinates for the same tRNA molecule in a different crystal form have been published recently by another group[11]. These coordinates are based on a refinement against X-ray data at 2.7-Å resolution. In contrast to our results, their ψ values show all the ribose groups to have the g^+ conformation about C5'–C4'. A second set of coordinates for the orthorhombic crystal form[12] does contain some sugars in conformations other than g^+, but generally not in the same places as we find. Although we cannot yet be certain that we have all our ribose conformations correct, most of them have been sustained during refinement. The correlation we have found between g^- and C2'-*endo*, with its simple physical explanation, argues strongly in favour of our interpretation.

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Production of nerve growth factor by the mouse adrenal medulla

PREVIOUS studies in our laboratories[1] have demonstrated that nerve fibres from explanted chick embryonic sensory ganglia become preferentially orientated towards fragments of mouse sarcoma 180 or adrenal medulla. It was suggested that this orientation was mediated by the release from the tissues of nerve growth factor (NGF). Direct experimental evidence for this hypothesis was not presented but the effect could be duplicated by using a capillary tube containing a solution of purified NGF instead of the tissue fragment, thus establishing that the protein could exert a directional effect on the growth of nerve fibres *in vitro*. Independently, Burnstock and coworkers[2] have reported similar orientation effects for rat sympathetic ganglia grown in proximity to a range of rat and other tissues. These results are potentially of considerable importance since they suggest that the orientation of peripheral nerves *in vivo* may be regulated, at least in part, by the release of NGF from the end organ. We report here the results of further experiments which confirm that NGF, as identified by biological activity and specific immunological properties, is produced and released by the adrenal medulla in culture.

Table 1 Orientation of nerve fibres towards and away from explants of adrenal medulla in tissue culture

Score	Towards adrenal	Away from adrenal
0	54	133
0.5	57	16
1	19	3
1.5	16	2
2	6	0
2.5	2	0

Frequencies with which the stated scores (on the accepted arbitrary scale) were obtained. From these data the mean scores ± s.e.m. are 0.57±0.05 (towards) and 0.09±0.02 (away). The frequency distributions are significantly different ($\chi^2 = 179$, $P < 0.001$). For experimental details, see text.

The orientation of nerve fibres promoted by the mouse adrenal medulla is evident from the results given in Table 1. Cultures were set up essentially as previously described[1] and the fibre outgrowth assessed on the accepted arbitrary scale[3]. The effect was investigated further by maintaining intact adrenal glands (4–5 per preparation, 578 total) from fully mature male mice (Swiss albinos) as organ cultures essentially by the method of Trowell[4]. The culture medium was changed at the half-way stage of the experiment (24 h) and the total medium collected and pooled. Parallel control experiments without tissue were carried out simultaneously. The recovered media were then concentrated (~ 20 times) by ultrafiltration (Minicon concentrators, 15,000 MW cutoff, Amicon). The adrenal glands were recovered at the end of the experiment and the water-soluble material was extracted and lyophilised. A comparable extract from freshly dissected glands (546 total) was also made. The samples were then tested for NGF activity in the standard bioassay[5,6]. The adrenal glands were found to contain no detectable activity before the organ culture but released biologically active material into the culture medium and thereafter contained significant levels of the factor. (Tables 2 and 3.)

The immunological properties of the active material were then examined to establish that the observed effects were due specifically to NGF and not to some other, general growth-promoting agent. Two main types of NGF have been isolated; those from mouse salivary gland and from snake venom[7,8]. The factors are immunologically distinct[9,10] as shown by different titres with homologous and heterologous antisera (see also Table 4). NGF of the immunological type found in snake venom is not confined to this species but occurs also in mammalian sera[9]. The active material from the mouse adrenal medulla was now found to have identical immunological properties (within the limitations of the test) to the NGF from mouse salivary gland (Table 4). Further, the orientation effect described above was blocked by similarly high dilutions of the antiserum to the salivary protein (Table 4).

Table 2 NGF activity in concentrated organ culture media from preparations of adrenal medulla

Score	Experimental	Control
0	12	79
0.5	34	3
1	22	0
1.5	7	0
2	1	0

Cultures were maintained in a water-saturated atmosphere of 95% oxygen and 5% carbon dioxide in a pre-selected medium (1–1.5 ml per preparation, all components from Gibco-Biocult) consisting of RPMI 1640 (80%), foetal calf serum (10%), tryptose phosphate broth (10%), penicillin (100 units ml^{-1}), streptomycin (100 μg ml^{-1}) and L-glutamine (2 mM). The table gives the frequencies with which the stated scores (on the accepted arbitrary scale) were obtained for the concentrated media cultured with (experimental) and without (control) tissue. From these data the mean scores ± s.e.m. are 0.68±0.05 (experimental) and 0.02±0.01 (control). The frequency distributions are significantly different ($\chi^2 = 432$, $P < 0.001$). For further details, see text.

These results do not, however, distinguish between the *de novo* synthesis of NGF in culture and the activation and release from the gland of a potential precursor. Such an activation might be achieved by proteolytic cleavage, possibly resulting from the action of a native esterase as has been tentatively suggested for the NGF from mouse salivary gland[11]. To test this hypothesis, a soluble extract of mouse adrenal glands (470 glands, 15% w/v in water) was incubated (37 °C, 24 h) in the presence and absence of the purified γ (esteropeptidase) subunit from the mouse 7S NGF (ref. 12) and some of the esterase-active and other fractions from the purification of the latter protein[13]. In no case was NGF activity detectable (at 0.5 mg ml^{-1} lyophilised extract) in bioassay. A further possibility, apparent in all studies on the biosynthesis of NGF *in vitro*, is that the tissue releases an ezyme which causes the formation of NGF from a precursor in the culture medium. This precursor would presumably be located in the serum component of the medium. The adrenal clearly does not normally contain such an enzyme, however, since the standard bioassay of the soluble extract (which gives negative results) involves incubation of the material in a tissue culture medium essentially similar to that used in the organ culture experiments. More importantly, the NGF detected in the present study is immunologically identical to the protein from mouse salivary gland and is therefore more likely to be derived from the mouse adrenal medulla than from foetal calf serum. We have shown previously[9] that the growth factors present in human and mouse sera are immunologically distinct from the salivary protein and Oger *et al.*[14] have reported that foetal calf serum does not contain immunoreactive NGF as judged by a highly sensitive phage assay based on this protein.

Table 3 NGF activity in soluble extracts of adrenal medulla before and after culture

Score	Cultured adrenals	Fresh adrenals
0	0	20
0.5	9	1
1	4	0
1.5	5	0
2	2	0

Glands (cultured or fresh) were homogenised in distilled water (30% w/v) and the extract centrifuged (48,000*g*, 60 min) and lyophilised. In general 1 g wet weight of tissue gave ~15 mg of lyophilised extract. Samples were assayed at a final concentration of 5 mg ml^{-1}: the table gives the frequencies with which the stated scores (on the accepted arbitrary scale) were obtained. From these data the mean scores ± s.e.m. are 1.00±0.12 (cultured) and 0.02±0.02 (fresh). The frequency distributions are significantly different ($\chi^2 = 27$, $P < 0.001$). In addition, preparations of microsomes (both before and after treatment with 1% Triton X-100 to liberate any membrane-bound protein) from uncultured adrenals were inactive. For further details, see text.

Table 4 Immunological properties of the NGF from mouse adrenal medulla

	Antibody	
Antigen	Mouse submaxillary gland NGF	*Vipera russelli* NGF
Mouse submaxillary gland NGF (purified)	6,000	15
Vipera russelli NGF (purified)	<3	6,000
Adrenal medulla, orientation effect	3,000	15
Adrenal medulla, concentrated organ culture medium	6,000	15

The maximum dilution of the appropriate antiserum which will block the effect of 1 BU of the NGF preparation (on the accepted arbitrary scale) or completely inhibit the production of nerve fibres in orientation experiments.

Our results then suggest that the level of NGF in the normal adrenal is < 1 biological unit (BU) per g wet weight, the limit detectable by bioassay under the conditions used. This finding is in sharp contrast to those of Hendry[15] and Johnson *et al.*[16] who obtained by radioimmunoassay values of 100–300 BU g^{-1}. To reduce the possibility of interference in the bioassay, a soluble extract (200 glands) was fractionated by ion exchange chromatography as previously reported for mammalian sera[9] but again no activity could be detected. A possible explanation of these differences is that the adrenal contains NGF in a form which is biologically inactive but capable of combining with antibody. To test this hypothesis, the homologous antiserum titre for salivary NGF was determined in the presence and absence of adrenal extract (0.5 mg ml^{-1}). The titres were the same in both cases, however, showing that the extract did not contain significant amounts of material capable of competing with NGF for antibody. According to the data from radioimmunoassay, a change in titre of up to tenfold would have been expected. These findings may reflect the reported difficulty[17] in obtaining NGF in sufficiently highly purified form to permit valid immunochemical studies. This is particularly relevant for studies based on radioimmunoassay, which is an extremely sensitive procedure.

There have been a number of reports on the synthesis *in vitro* of NGF by cell lines and tissues[14,18–22]. We believe, however, that we are the first to demonstrate the production of NGF *in vitro* by an organ that does not normally contain the protein *in vivo*. Our findings may relate to those of Hendry and Iversen[23] who showed that after sialectomy in the mouse, which apparently removes the major site of synthesis of NGF in the animal, other unidentified organs begin to secrete the factor. The adrenal gland may be such an organ. Alternatively, our findings may imply that the adrenal begins to synthesise NGF in culture in response to the artificial conditions of the experiment. In this event, any attempt to extrapolate from the production of NGF *in vitro* to the situation *in vivo* must be made with great caution. This may be particularly relevant since preliminary studies show that the synthesis of NGF in culture is not restricted to the adrenal medulla but may be a general property of mouse and rat tissues.

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A paradox in the lateralisation of the visual evoked response

THE average evoked response in man to stimulation of the visual field with a reversing checkerboard pattern and recorded from the scalp over the occipital region has a waveform which is consistent both within and between subjects. The most characteristic feature for full-field stimulation is a major positive component at about 100 ms which is recorded maximally 5 cm above the inion in the midline and is distributed fairly symmetrically over both sides of the head (Fig. 1). The pathways from the retina to the visual cortex undergo partial decussation in the chiasma, so that information presented to the left half of the visual field passes to the right hemisphere, whereas the left hemisphere receives signals from the right half of the visual field. Consequently, it might be predicted that stimulation of one half field will produce an evoked response which is maximal over the contralateral hemisphere. The data presented here show that, contrary to this prediction, the maximal response occurs at the scalp electrodes situated over the hemisphere ipsilateral to the field stimulated.

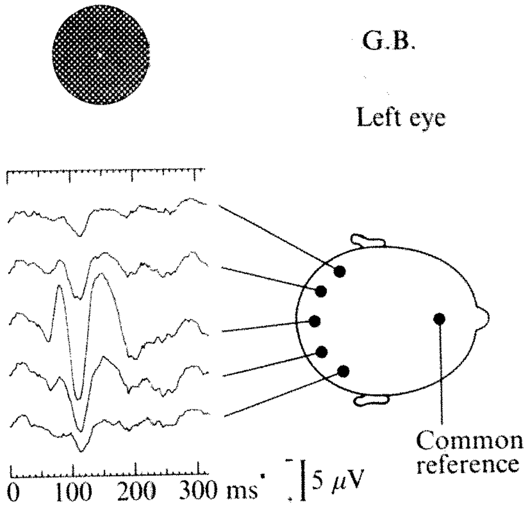

Fig. 1 Monopolar recording of the response to stimulation of the whole field (out to 16°) for the left eye of subject G.B. The positive large component at about 110 ms is seen maximally at the midline electrode and falls off fairly symmetrically in amplitude on the two sides of the head. Time scale in 10, 50 and 100 ms marks.

The average evoked response to stimulation of the left and right half fields for each eye of nine healthy individuals (age range 19–29 yr) was recorded from a chain of chlorided silver cup electrodes 5 cm up from the inion. The central electrode of the chain was in the midline and the other electrodes were spaced 5 cm and 10 cm laterally on either side. Each electrode was referred to a common midfrontal electrode 12 cm above the nasion and in the midline. A black-and-white checkerboard pattern was back-projected on to a translucent screen which was masked to produce a semicircular field extending 16° out from the fixation point for a subject sitting 1 m from the screen. The subject was instructed to fixate a constantly illuminated spot of light at the centre of the vertical diameter of the stimulus field. Each square of the checkerboard subtended an angle of 50′ and the brightness of the white squares as seen by the subject was 227 cd m^{-2}, whereas that of the black squares was 8.2 cd m^{-2}. The checkerboard pattern was reversed twice per second and the electrical activity at the five electrodes was sampled at 400 Hz for 320 ms after each reversal. The average evoked response for 200 complementary pattern reversals was recorded with a PDP-12 computer.

The monopolar records in Fig. 2 show that, paradoxically, the response to stimulation of a half field is recorded maximally from the midline and from electrodes over the hemisphere ipsilateral to the field of stimulation, whereas the response on the contralateral side is comparatively flat. This ipsilateral positivity was recorded consistently in every subject for each half field and for stimulation of either eye. The bipolar records in Fig. 2 show the distribution of responses recorded with the five occipital electrodes connected as a bipolar chain rather than linked to a common midfrontal reference; the maximal response is now apparently recorded over the contralateral hemisphere. Comparison of

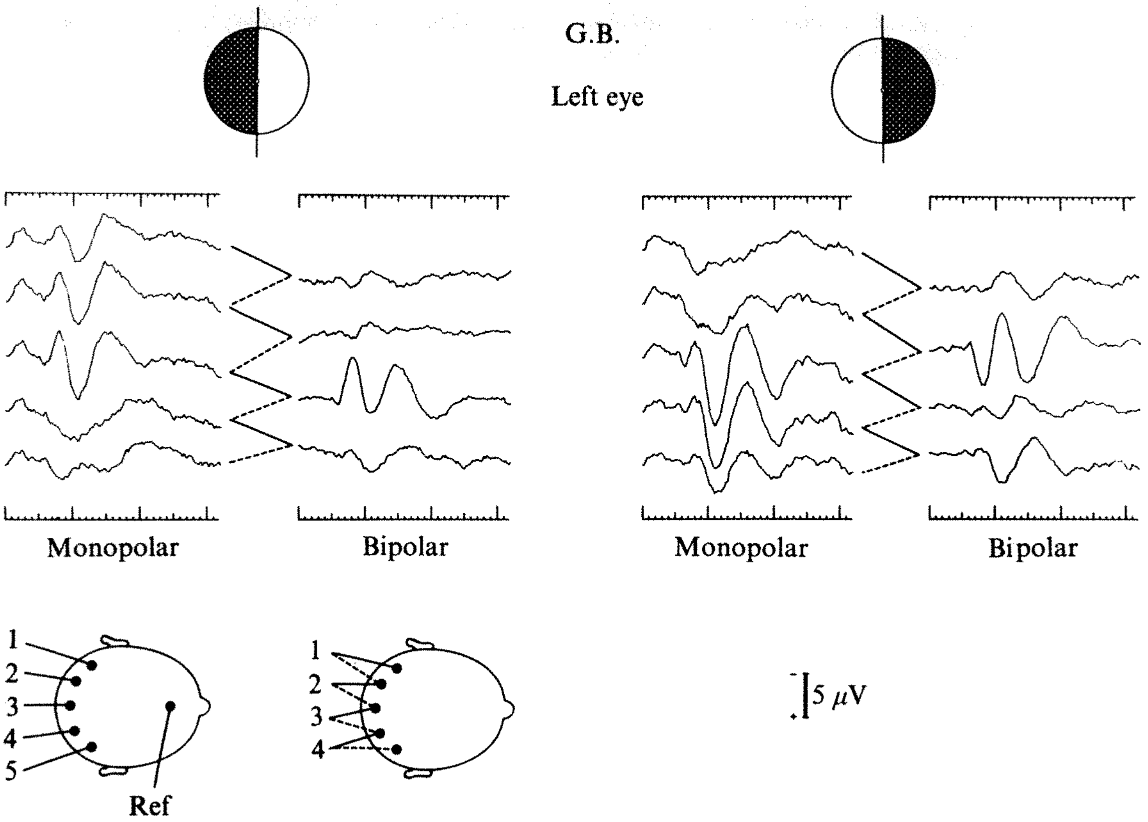

Fig. 2 Monopolar and bipolar records for stimulation of the two half fields for the left eye of subject G.B. In the monopolar record the large positive component at about 110 ms is seen at the midline electrode and at the electrodes ipsilateral to the stimulated half field, whereas the contralateral channels are relatively flat. In the bipolar record, which reflects voltage gradient, the ipsilateral channels show little or no response, whereas the midline–contralateral channel shows the maximum response.

the monopolar and bipolar records, however, shows that it is erroneous to conclude from such a bipolar record that the response is largest contralaterally; although the maximum voltage gradient is indeed between the midline electrode and its neighbour over the contralateral hemisphere, it is the response at the midline electrode that has the greater amplitude, as the monopolar record shows. The bipolar recording shows a comparatively flat response on the ipsilateral side, not because there is little or no activity, but because there is equally high amplitude activity at all electrodes on this side of the scalp. Similarly, on the contralateral side, there is low amplitude activity at both electrodes, not the high amplitude activity that would be expected if the maximal response occurred over this hemisphere.

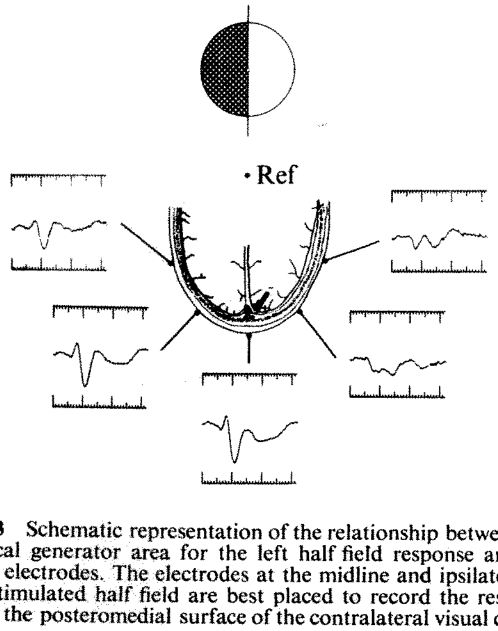

Fig. 3 Schematic representation of the relationship between the cortical generator area for the left half field response and the scalp electrodes. The electrodes at the midline and ipsilateral to the stimulated half field are best placed to record the response from the posteromedial surface of the contralateral visual cortex.

A possible explanation of this unexpected lateralisation of the evoked response is that the cortical generator areas for the pattern stimulus are largely situated on the medial and posteromedial surface of the visual cortex where the neurones are transversely oriented. Thus, electrodes over the hemisphere ipsilateral to the field stimulated are optimally placed to record the response from these generator areas, whereas electrodes over the contralateral hemisphere are not so placed. The schematic representation of this hypothesis in Fig. 3 shows that the electrodes on the left side of the head are best placed to record a response from the medial surface of the right visual cortex evoked by pattern stimulation of the left half field. The electrodes over the right hemisphere, however, are approximately perpendicular to the axis of the generator area and therefore record very little response.

The paradoxical distribution of the evoked response to stimulation of the visual half fields has important implications for the interpretation of recordings made in cases of hemianopia. The findings in healthy subjects can be used to predict the type of response asymmetry to be expected in cases of either homonymous or bitemporal hemianopia. In homonymous hemianopia the asymmetry of the evoked response recorded to whole-field pattern stimulation is the same for both eyes ('uncrossed' asymmetry) and the 'blind' field will be contralateral to the side over which the response is maximally recorded. In bitemporal hemianopia (that is, where the fibres from the nasal retina of each eye are damaged) the asymmetry is opposite for the two eyes ('crossed' asymmetry) and the maximal response will be seen on the right side for the left eye and on the left side for the right eye. Recordings made from patients with hemianopia support these predictions[1].

In the present experiments a half field stimulus extending out to an eccentricity of 16° has been used, but a similar ipsilateral distribution of the major positive component of the pattern reversal response can also be recorded when stimulating smaller segments of each half field. It is still clearly demonstrable when only the central 0–4° are stimulated. When stimulation is confined to the macular area itself (0–2°), however, the response becomes less well lateralised and may even have a contralateral predominance. This is what would be predicted on the scheme illustrated in Fig. 3, as the cortical representation of the macula is at the posterior pole of the occipital lobe and the generator neurones are here facing posteriorly rather than medially.

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Synaptic vesicle recycling in synaptosomes *in vitro*

PINCHED-OFF presynaptic nerve terminals (synaptosomes) prepared from rat brain homogenates[1,2] retain many of the functional properties of intact neurones[3,4]. They can accumulate K$^+$ and extrude Na$^+$ against concentration gradients[5,6], and their membranes seem to develop K$^+$ diffusion potentials which behave like the membrane potentials of most neurones[7]. Furthermore, the isolated terminals exhibit an increased rate of Ca^{2+} accumulation[8] and Ca^{2+}-dependent transmitter release[8–10] when exposed to depolarising agents such as veratridine or an increased external K$^+$ concentration ([K]$_0$). The latter findings are consistent with the 'calcium hypothesis' of depolarisation–secretion coupling[11]. The calcium which enters the terminals during depolarisation seems to trigger the release of transmitter from intraterminal vesicles by an exocytotic process involving transient fusion of the vesicle membrane with the surface membrane[12–14]. This hypothesis is supported by experiments with electron-opaque extracellular markers; the appearance of the markers within intraterminal vesicles, after stimulation, has been taken as evidence for vesicle recycling[15–17]. Here we report similar observations on synaptosomes incubated *in vitro*, showing that synaptosomes function like intact terminals in this respect as well.

Synaptosomes were prepared from whole rat brain homogenates by a slightly modified[18] Gray and Whittaker procedure[2]. The standard ('Na+5K') synaptosome incubation solution contained (mmol l^{-1}): NaCl, 145; KCl, 5; CaCl$_2$, 1.2; MgCl$_2$, 1.3; NaH$_2$PO$_4$, 1.2; glucose, 10; HEPES, 20; the solution was titrated to *p*H 7.6 at 20 °C with Tris base. In some instances, part of the NaCl was replaced mol-for-mol by KCl (='K-rich' incubation media; see below).

Synaptosomes from the sucrose gradient[2] were equilibrated[7] with ice-cold Na+5K, and centrifuged at 9,000*g* for 5 min at 4 °C. The supernatant was discarded, and the pellets resuspended in Na+5K (approximately 2 mg synaptosome protein ml^{-1}) and incubated for 12 min at 30 °C. Additional aliquots of Na+5K or of K-rich solution (to give a final [K]$_0$ of 35 or 50 mM) were then added. In most experiments the incubation solutions also contained sufficient colloidal thorium dioxide (Thorotrast, a 25% suspension, from Fellows Testagar, Anaheim, California, a gift from Dr A. Enders) to give a final concentration of 3.3%, or horseradish peroxidase (HRP, type VI, Sigma, St Louis, Missouri, previously dialysed[15] against Na+5K) to give a final concentration of 5 mg ml^{-1} after dilution.

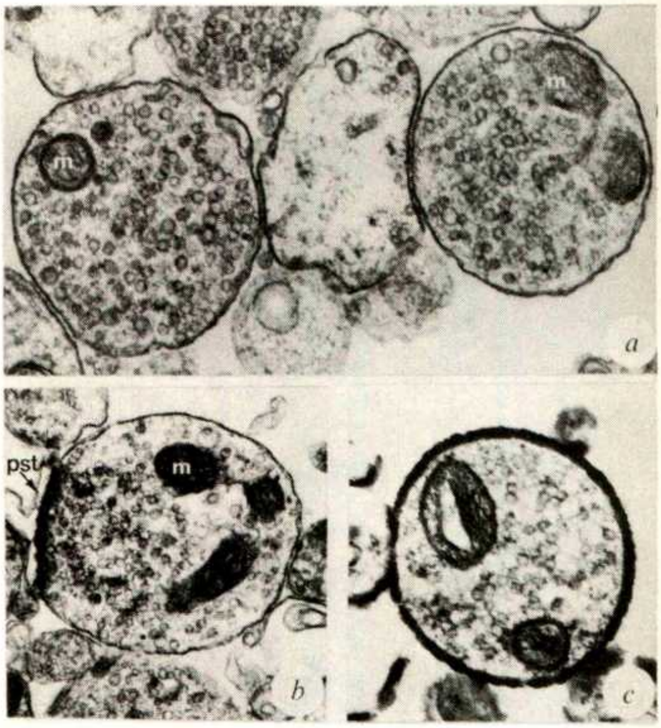

Fig. 1 Morphology of synaptosomes incubated in standard Na+5K. *a*, Vesicle-filled terminals with intraterminal mitochondria (m). No extracellular marker present. ×43,000. *b*, Similar to *a*, with a clearly demarcated postsynaptic thickening (pst). ×33,000. *c*, Synaptosome fixed and stained after 30 min incubation in Na+5K containing 5 mg ml^{-1} HRP. Note halo of HRP reaction product around synaptosome, and absence of product in the cytoplasm. ×39,000.

The diluted suspensions were incubated at 30 °C for an additional 2–30 min. Aliquots were then diluted with one volume Na+5K plus two volumes Karnovsky's cacodylate-buffered fixative[19], and refrigerated overnight; the samples were then centrifuged, and the supernatant solutions discarded. The HRP-treated pellets were reacted with H$_2$O$_2$ and diaminobenzidine[16] for 2 h at room temperature, and washed several times in cacodylate buffer[16]. All pellets were stained with potassium ferrocyanide-reduced osmium tetroxide[20], followed by osmium tetroxide. They were dehydrated in increasing concentrations of ethanol and embedded in Araldite. Thin sections were examined electron microscopically.

Most synaptosomes appeared more-or-less circular in cross section (Figs 1 and 2). Those fixed after incubation in Na+5K were generally filled with uniform-sized vesicles (about 400–500 Å in diameter; Fig. 1*a–c*), although occasional larger vesicles were seen. The terminals also usually contained mitochondria (Fig. 1). When exposed to HRP, the terminals incubated in Na+5K were surrounded by a halo of HRP reaction product (Fig. 1*c*); the apparent lack of HRP penetration into the cytoplasm implies that the surface membrane had resealed. In some terminals (see Fig. 2*c*), however, the extracellular marker was distributed diffusely throughout the cytoplasm, but was excluded from the synaptic vesicles; this presumably occurred in terminals which had not completely resealed, or which were subsequently damaged. The morphological markers may therefore be useful for the quantitative assessment of synaptosome integrity.

After exposure to media containing 35 or 50 mM K, the intraterminal vesicles appeared much more variable in size, and large vacuoles (cisternae[16]) were found in many terminals (Fig 2*a* and *c*). Coated vesicles[16,21] were observed in some of the terminals exposed to elevated $[K]_0$, (Fig. 2*b*). Even after prolonged (15–30 min) incubations in K-rich media, the terminals were not markedly depleted of vesicles.

When incubated in HRP-containing K-rich media, vesicles filled with electron-opaque material (presumably HRP reaction product) were seen in some of the terminals; this is illustrated in Fig. 2*b*, which also shows a coated vesicle filled with HRP reaction product. This latter finding fits with the hypothesis[16,21] that one of the steps in the vesicle retrieval process may involve coated vesicles. Further evidence of synaptic vesicle recycling was obtained with Thorotrast-treated terminals, where both vesicles and large vacuoles contained the marker even after a short period of incubation in a depolarising solution[22] (Fig. 2*c*).

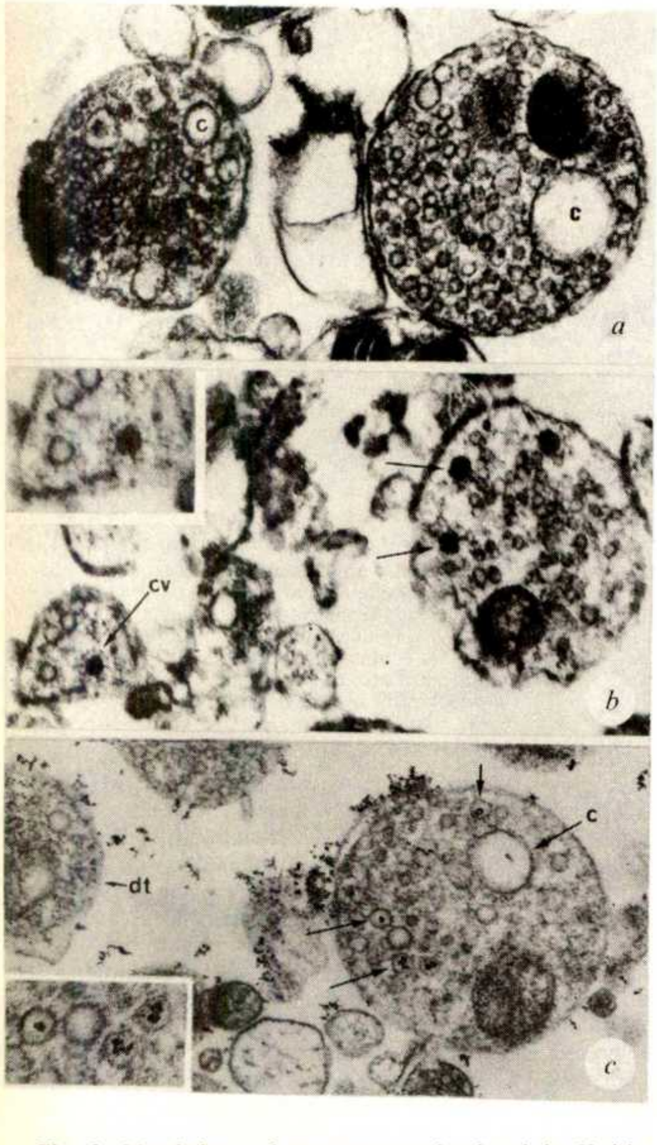

Fig. 2 Morphology of synaptosomes incubated in K-rich media. *a*, Synaptosomes incubated in 100 Na+50K solution, without extracellular marker for 15 min. Note marked heterogeneity of synaptic vesicle size and presence of large vacuoles (c). ×41,000. *b*, Synaptosomes incubated in 100 Na+50K containing 5 mg ml^{-1} HRP, for 30 min. Note HRP-labelled coated vesicle (cv); unlabelled arrows indicate two other HRP-labelled synaptic vesicles. ×45,000. Inset: coated vesicle at ×71,000. *c*, Synaptosomes incubated for 2 min in 115 Na+35K containing 3.3% Thorotrast. Unlabelled arrows indicate some of the Thorotrast-containing vesicles. A Thorotrast-containing large vacuole (c), and a damaged terminal (dt) with diffusely distributed Thorotrast are also seen. ×50,000. Inset: higher magnification of Thorotrast-labelled vesicles. ×88,000.

We have repeatedly observed that the tracer-labelled vesicles (Fig. 2*b* and *c*) are usually larger than most vesicles in 'control' synaptosomes (Fig. 1). Preliminary evidence (unpublished) indicates that the initial step in membrane retrieval seems to involve the pinching-off of these large vesicles from the plasma membrane. The further implications of large vesicle formation are being investigated.

Our observations are consistent with a mechanism involving transient plasma membrane fusion and recycling of synaptic vesicles. They suggest that synaptosomes behave like intact terminals with respect to the mechanism by which they release vesicular contents. Thus, synaptosomes may be useful for the study of transmitter release and vesicle recycling processes in the central nervous system.

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Extremely low frequency, weak electric fields affect schedule-controlled behaviour of monkeys

THE effect of weak electric fields on behaviour of orgasnims has potentially important implications for basic neurophysiology and evaluation of environmental hazards (chronic exposure to weak 60-Hz electric fields has become a way of life). We have demonstrated[1] that weak (approximately 7 V m^{-1} p–p), extremely low frequency (7 Hz) electric fields could modify a lever-press response in monkeys. We summarise here data[2] confirming that finding for a larger group of animals with more replications and presenting evidence for a dose-dependent relationship for

7-Hz fields. In addition there are indications of a threshold of 10–56 V m^{-1} p–p for field frequencies higher than electroencephalogram (EEG) range (1–32 Hz). Adey[3] has hypothesised that since these field levels are clearly too low to trigger synapses in the fashion prescribed by classical neurophysiology, more subtle membrane sensitivities may be functioning as central nervous system mediators of the observed behavioural effects. One possible mediator is the degree of calcium efflux across the membrane[4].

We have explored possible behavioural effects of fields at frequencies from 7–75 Hz and voltages from 1–56 V m^{-1} p–p, together with a preliminary study at 100 V m^{-1} p–p. Five female *Macaca nemestrina* monkeys (3–6 kg) were trained on a Skinner[5] conditioning schedule. The animals had to wait 5 s between lever presses and to press within a 2.5 s 'limited hold'; each response recycled the 5-s delay period—an inter-response time (IRT) schedule (IRT > 5 s; < 7.5 s). The analysis of behaviour in terms of IRT has been widely used to evaluate behavioural effects of low doses of drugs (for example, ref. 6). For details, see Fig. 1, which shows an example of the histogram of IRTs across three voltage levels (1, 10 and 56 V m^{-1} p–p) for the 7-Hz condition.

Our data analyses are limited to voltage levels of 1–56 V m^{-1} p–p because preliminary data at the 100-V m^{-1} level suggested that fields of this strength affected the randomly interspersed control days—that is, there seemed to be a 24-h 'carry-over' effect. Evaluation of possible effects at this level awaits further experimentation.

For fields with voltages from 1–56 V m^{-1}, the *a priori* hypothesis of shortened IRTs was tested with t tests (Fig. 2). Since our previous data[1] had indicated that low voltage ($\sim$7 V m^{-1}) low frequency (7-Hz) fields resulted in shorter times between responses than control no-field conditions, it was possible to examine the present data with this specific statistical hypothesis. At 1 V m^{-1} none of the observed differences between field and no-field mean IRT$\pm$s.d. was statistically significant. There is evidence for a frequency-specific (7-Hz) threshold at 10 V m^{-1} p–p. The difference observed is small ($\sim$0.1 s) but significant at the 0.05 level. This low threshold for 7 Hz may reflect the fact that this frequency is in the range of normal brain wave activity (hippocampal theta) for performing monkeys. At 56 V m^{-1}, both 7-Hz and 75-Hz fields produce large ($\sim$0.3 s) and statistically significant reductions in IRT. Note that these data suggest a dose-dependent relationship for 7-Hz fields; mean IRT differences increase systematically across the three voltage levels from 0.07 s at 1 V m^{-1} to 0.12 s at 10 V m^{-1} to 0.29 s at 56 V m^{-1}.

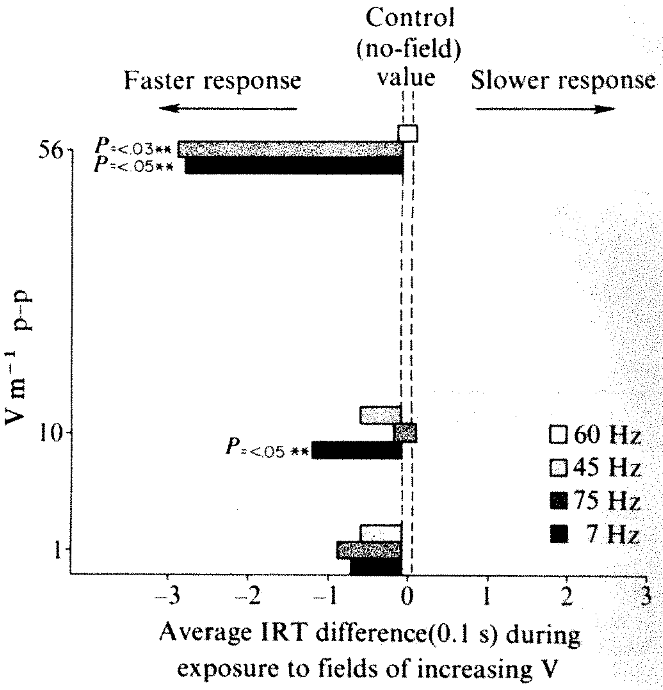

Fig. 2 Faster response (reduced IRT) as a function of frequency and voltage of applied electric field. Asterisks denote statistically significant shifts. All data are based on four repetitions of each experiment for each of 4–5 monkeys. Note the low voltage threshold (10 V m^{-1}) for 7 Hz, a frequency which is relevant to normal brain activity.

These results substantiate our earlier finding that the 7-Hz field was associated with a very low voltage threshold of

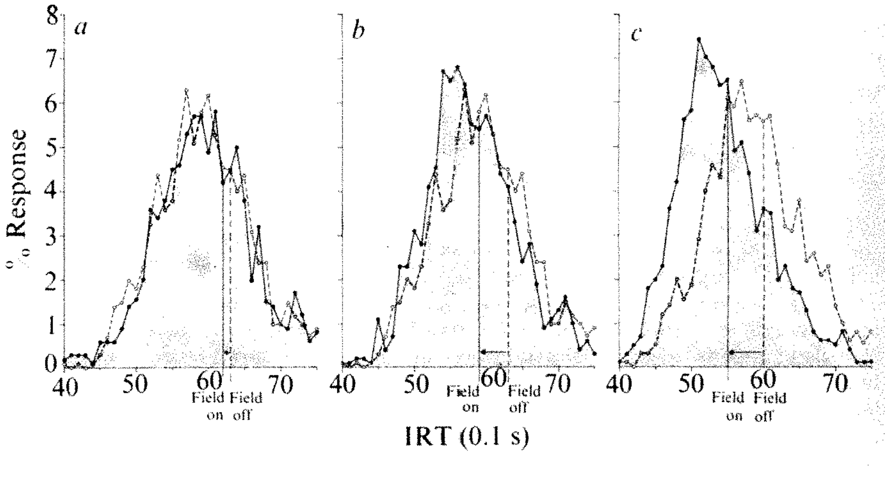

Fig. 1 An example of the IRT distributions observed for one 4-h experiment for one unimplanted animal at 1 (*a*), 10 (*b*) and 56 V m^{-1} p–p (*c*). Field frequency = 7 Hz. Exposure to this field resulted in a small shift towards faster IRTs at 10 V m^{-1} and a much larger shift at 56 V m^{-1}. The average difference observed over all monkeys for this field configuration was 0.07 s at 1 V m^{-1}, 0.1 s at 10 V m^{-1} and 0.3 s at 56 V m^{-1}. Field (●) and no-field (○) distributions are contrasted. Number of responses and $\bar{x}$ for field on: *a*, 1,024 and 62; *b*, 1,026 and 59; *c*, 973 and 55. Field off: *a*, 1,505 and 63; *b*, 1,505 and 63; *c*, 987 and 60. Histograms for both field and no-field conditions are a good approximation of the normal distribution. Four such histograms were generated for each frequency–voltage combination for each animal. About 300 field and no-field experiments were carried out. Correct responses were reinforced with small quantities of fruit juice; water deprivation was used during the initial stages of training but was not necessary during testing. No external cues (lights, bells) signalled availability of the reinforcer; the animal was required to respond to the lapse of time following the occurrence of its own preceding response. After 80–100 d training, stable performance (70–80% correct) was achieved and a random series of field/no-field tests carried out. Three animals were implanted with EEG electrodes and two were not, so that possible 'antenna' effects due to the presence of the electrodes could be assessed. Four 4-h replications of each field condition and each control condition (no-field) were analysed for each monkey. Field conditions included frequencies of 0, 7, 45, 60 and 75 Hz, and voltage levels of 0, 1, 10, 56 and 100 V m^{-1} p–p. One animal died from accidental causes at the conclusion of the 10 V m^{-1} experiments so n was reduced to 4 for the experiments at 56 and 100 V m^{-1}. Experiments were carried out in a double-bronze mesh screened enclosure; a.c. conductors (except for a shielded camera) were excluded. Monkeys sat in plastic chairs between parallel field plates (1 m^2 50 cm apart) centred in the enclosure. The fields were measured with a specially devised fibre optic probe, and were found to be satisfactorily uniform and balanced[7]. Current from the imposed field flowing to ground through the implanted electrodes was measured in a phantom head at slightly less than 0.9 nA at 10 V m^{-1} p–p. IRT was tape recorded and analysed by computer in 0.1-s intervals. Responses longer than 17.5 s were excluded to avoid skewing the distribution with occasional very slow responses.

Table 1 Summary of t tests for mean IRTs ±s.d. (no-field minus field differences, across voltage levels)

Voltage (V m^{-1} p–p)	Frequency (Hz)	No. of monkeys	Average no. of responses	Mean difference ±s.e.d. (10th s)	P	s.d. difference ±s.e.d. (10th s)	P
1	7	5	3,299	0.70±0.69	<0.20	0.12±0.97	<0.50
	45	5	4,229	0.55±1.35	<0.40	−0.28±1.04	<0.45
	75	5	3,360	0.94±0.62	<0.15	−0.20±0.66	<0.40
10	7	5	3,560	1.18±0.48	<0.05*	1.60±0.52	<0.03*
	45	5	3,982	0.55±0.61	<0.25	0.10±0.41	<0.45
	75	5	3,560	0.00±0.60	—	−0.12±1.06	<0.50
56	7	4	2,764	2.88±1.27	<0.05*	1.78±0.35	<0.01*
	60	4	2,662	−0.03±0.70	<0.50	0.58±0.28	<0.10
	75	4	2,862	2.90±0.72	<0.03*	2.25±0.69	<0.03*

*Statistically significant shifts.

approximately the same level (7 V m^{-1} previously and 10 V m^{-1} here) and indicate much higher thresholds for frequencies which are not relevant to normal brain wave activity. For 75 Hz, the threshold is between 10 and 56 V m^{-1}; for 60 Hz it may be higher.

Numerical data and t tests for the mean IRT±s.d. for voltage levels from 1–56 V m^{-1} p–p are summarised in Table 1. No effect is observed at 1 V m^{-1}. At 10 V m^{-1}, the 7-Hz field was associated with a significantly smaller s.d. At 56 V m^{-1} all field frequencies were associated with s.d.s smaller than the no-field values. These differences were statistically significant for 7 and 75 Hz, and reach a borderline level ($P<0.10$) for 60 Hz.

Data for individual animals reflect the orderliness of the average values. At 1 V m^{-1} the mean IRTs varied randomly across the field conditions. At 10 V m^{-1}, the 7-Hz condition was associated with the fastest mean IRT and smallest s.d. of all field conditions for every animal tested. At 56 V m^{-1} all field-associated s.d.s were smaller than the no-field condition for every animal. All mean IRTs, except those associated with the 60-Hz field, were faster than no-field means at 56 V m^{-1} for every animal tested.

Table 2 Analysis of variance of implanted compared with unimplanted animals at 1 and 10, 56 and 100 V m^{-1}

		Mean IRTs ±s.d.					
Voltage		1 and 10 V m^{-1}		56 V m^{-1}		100 V m^{-1}	
Source		F	P	F	P	F	P
Group	$\bar{x}$	0.048	0.84	0.770	0.47	0.708	0.49
	σ	0.378	0.58	0.014	0.92	1.469	0.35
Replication	$\bar{x}$	0.953	0.46	0.769	0.55	0.497	0.70
	σ	0.412	0.75	0.865	0.51	2.434	0.16

Analysis of variance of IRTs for implanted compared with unimplanted animals revealed no significant effects (Table 2). Thus, it is improbable that the observed effects can be attributed to the presence of EEG recording electrodes in the brain (the so-called 'antenna' hypothesis). Additional analyses of variance were carried out for IRT data across frequency and voltage levels to look for possible information secondary to the initial effect of shortened IRTs. These analyses revealed no unexpected findings. Field exposure contributed significantly to the variance of the s.d.s of mean IRTs at low voltages (1 and 10 V m^{-1} exposures, $F=2.59$, $P<0.05$), but not to the average IRTs —probably because of the small n. At 56 V m^{-1} both s.d.s and average IRTs were associated with significant F values ($F=5.75$, $P<0.02$ and $F=5.22$, $P<0.03$, respectively). Replications did not contribute significantly to the variance at any of the voltage levels tested.

Ethological studies of electric and magnetic fields lend support to the behavioural data presented here. Keeton[8] demonstrated that weak magnetic fields can affect the homing of pigeons; certain species of fish utilise weak electric fields to detect their prey[9]; and 10-Hz electric fields (25 mV cm^{-1}) can alter circadian rhythms of activity in man[10]. Laboratory studies to date have provided only equivocal evidence[11-15]. Absence of a clear-cut picture may be related to specific attributes of the separate experiments in such factors as field frequency, voltage, duration of exposure and behavioural assay. Keeton's pigeons ignored the presence of an imposed magnetic field if the Sun were available as a homing cue. Similarly, weak electric fields may not affect performance on demanding behavioural tasks with multiple stimuli inherent in them. An animal carrying out such a task may be able to 'ignore' the presence of the field.

On the basis of studies on calcium binding in cerebral tissues[16,17], which have paralleled the present studies on behavioural effects of weak electric fields, and taking into consideration the physical model conceived by Grodsky[18], we propose that the potential for sensitivity to weak electric fields exists in all organisms whose nervous system is characterised by: slow electrical waves in the extracellular space; and a system of macromolecular sensors on the membrane surface which is capable of transducing components of the extracellular field by altered ion binding and an associated conformational change. In animals in which field detection is not intimately linked to survival, sensitivity can probably only be revealed in innocuous experiments of the kind described above which are characterised by long exposure duration, little external stimulation and low motivational requirements.

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Antivenom activity of rattlesnake blood plasma

MANY species of venomous snakes have a high resistance to their own venoms and to those from other individuals of the same species and other species[1–4]. The resistance is not absolute but it increases as the size of the snake increases, and varies also with the type of venom. Blood plasma or sera of several species neutralise the venom of the same species and of other species both *in vivo* and *in vitro*[5–11]. As Clark and Voris[10] reported, the whole serum of the eastern diamondback rattlesnake (*Crotalus adamanteus*) neutralises lethal doses of *C. adamanteus* venom in mice and the protective capacity of the serum is associated with the serum albumin fraction. Mixtures of *C. adamanteus* serum and venom show no evidence of precipitation, such as occurs when serum from animals hyperimmunised with *C. adamanteus* venom is mixed with *C. adamanteus* venom. The nature of the factor(s) in rattlesnake plasma, which neutralise rattlesnake venom toxins, and the mechanism of neutralisation are unknown. We have found that both western and eastern diamondback rattlesnake (*C. atrox* and *C. adamanteus*) blood plasma contain antivenom factor(s) and have a neutralisation capacity (NC), not only for *C. atrox* venom but also other rattlesnake venoms. The antivenom activity of *C. atrox* plasma is sufficiently potent, compared with commercial immunoglobulin antivenin, to be potentially useful in the management of snakebite.

Before testing for *C. atrox* plasma antivenom activity, we heated the plasma at 56 °C for 30 min to detoxify it[12]. The intraperitoneal toxicity (LD_{50}) of unheated *C. atrox* plasma in mice varied from 2.6 mg per 20 g mouse to 9.0 mg per 20 g mouse for plasma from 25 individual specimens of *C. atrox*. Pooled plasma (LD_{50}, 5 mg per 20 g mouse) from 100 captive *C. atrox* was used. Heating removed the toxicity ($LD_{50} > 40$ mg per 20 g mouse) and all subsequent experiments were carried out with heat-treated plasma. Biuret[13] assay was used to estimate protein content of plasmas and venoms and showed a protein content of about 40 mg ml^{-1} or about 50 mg ml^{-1}, when corrected for dilution by citrates for pooled *C. atrox* plasma. All antivenom and toxicity assays were done on a protein weight basis.

We found that both *C. adamanteus* and *C. atrox* plasma were potent antivenoms for several rattlesnake venoms (Table 1). The neutralisation capacity of both plasmas was compared with that of commercial antivenin by injecting mice with 5 mg of plasma protein or antivenin intraperitoneally and determining the LD_{50} of the venom in the antivenom-treated mice compared with that of untreated mice. Both plasmas had significantly higher neutralisation capacity values for various rattlesnake venoms than did antivenin, except in the case of South American rattlesnake venom (*C. durisus terrificus*) and the mohave rattlesnake venom (*C. scutulatus scutulatus*, brown variety). We found two varieties of *C. scutulatus scutulatus* readily distinguished by the toxicity of their venoms and the capacity of antivenoms to neutralise each venom. The two varieties are distinguished also by their colour. The yellow variety has more toxic venom and lower neutralisation capacity values for antivenoms than the brown variety.

Rattlesnake venoms are neutralised also when premixed *in vitro* with either rattlesnake plasma or commercial antivenin. A visible precipitate occurs with immunoglobulin antivenin but not with plasma.

To test the hypothesis that the antivenom activity is due to antigenic protein or multiple proteins in *C. atrox* plasma, rabbits (New Zealand White) were injected with heat-treated *C. atrox* plasma and Fruend's complete adjuvant and the resultant antisera were collected 1 week after a booster injection of *C. atrox* plasma and adjuvant 4–6 weeks after the initial injection. The antivenom activity of *C. atrox* plasma was removed completely when a 5-ml sample was treated three times with 1 ml of antisera and the resultant precipitates were removed by centrifugation. Addition of unimmunised rabbit serum to *C. atrox* plasma did not diminish the antivenom activity.

Clark[10] found the antivenom activity of *C. atrox* serum in the albumin fraction after chromatography on Sephadex G-100. Our results of polyacrylamide gel electrophoresis (PAGE)-immunodiffusion studies indicate that the antivenom activity is due not to albumin or some small molecule bound to albumin, but to separate antigenic proteins in the plasma.

Plasma proteins were fractionated on gradient (4.5–8% acrylamide) gel slabs using the discontinuous basic buffer system described by Ortec Inc. Each slab was cut into three sections and minced; the protein was extracted into saline and filtered through a 1-μm Millipore filter. Extract from each gel section was assayed for antivenom activity. The

Table 1 Capacity of four antivenoms to neutralise lethal toxicity of rattlesnake venoms

Venom	Neutralisation capacity			
	Pig plasma	Antivenin	*C. adamanteus* plasma	*C. atrox* plasma
C. adamanteus	1.3	4.5	7.7	6.5
C. atrox	1.4	2.7	3.5	4.3
C. viridis concolor	1.3	6.4	9.8	9.0
C. durisus terrificus	1.1	9.5	8.1	8.3
C. scutulatus scutulatus, brown	1.3	10.2	9.0	10.1
C. scutulatus scutulatus, yellow	1.1	2.7	5.8	6.4
Naja naja kaouthia	1.1	1.0	1.0	1.0

Venom lethal toxicity (LD_{50}) was assayed using Swiss–Webster mice (18–20 g). Twenty to thirty mice were used for each assay and the LD_{50} dose was calculated according to the method of moving average interpolation[14–16]. Venoms from rattlesnakes and cobras were obtained from our captive specimens by mild electrical stimulation of the mandibulae compressor glandulae muscles surrounding the venom gland[17]. Venoms from several healthy individuals, strictly of the same species, were pooled and centrifuged to remove cellular debris, then lyophilised and stored at −20 °C as a batch. Venoms handled in this manner can be stored for years without measurable loss of lethal toxicity. Neutralisation capacity is the ratio of the intraperitoneal LD_{50} of venom in mice treated with antivenom (5 mg protein given intraperitoneally) to the intraperitoneal LD_{50} of venom in untreated or saline-injected mice. A neutralisation capacity of 1 means that the antivenom had no effect on the LD_{50} of venom in mice in 24 h. The greater the neutralisation capacity the more potent was the antivenom. Pig plasma was obtained from a local slaughterhouse from blood collected in trisodium citrate (3.8%, 4 parts blood: 1 part citrate). Antivenin was Crotalidae polyvalent, equine, North and South American antisnakebite serum (Wyeth). *C. atrox* and *C. adamanteus* plasma were obtained from specimens in our serpentarium, either by heart puncture under anaesthesia[18] or by decapitation. The plasma was separated from individual citrated bloods and then pooled, centrifuged and stored frozen (−20 °C). Plasma was taken and stored in this manner to avoid serum clots. The antivenom activity of plasma and serum was the same.

Table 2 Effectiveness of *C. atrox* plasma compared with antivenin on mean survival times of New Zealand White rabbits (~2.7 kg) injected with *C. atrox* venom (6 mg kg^{-1})

Treatment	Antivenom pretreatment time (h)							
	−12	0	24	48	72	96	120	144
None	22±4	22±4	—	—	—	—	—	—
Pig plasma (1,000 mg)	20±4	22±4	22±4	—	—	—	—	—
Antivenin (200 mg)	24±4	30±4	24±4	—	—	—	—	—
C. atrox plasma (heated) (200 mg)	24±4	S	32±4	22±4	—	—	—	—
Antivenin (400 mg)	26±4	50±4	22±4	—	—	—	—	—
C. atrox plasma (heated) (400 mg)	26±4	S	45±5	28±4	22±4	—	—	—
Antivenin (800 mg)	26±4	S	22±4	—	—	—	—	—
C. atrox plasma (heated) (800 mg)	26±4	S	S	S	S	S	50±8	25±5

Each group consisted of three rabbits. Antivenom was injected intravenously either at the same time as the venom (intramuscularly) (that is pretreatment time = 0) or before the injection of venom at several pretreatment times (24–144 h). The first column minus 12 h (−12) refers to injection of venom 12 h before injection of antivenom. S indicates that all animals survived for several weeks until they were killed. Survival times are in hours.

activity was found in the middle gel cut separate from albumin. Analytical PAGE of the extract revealed four protein staining (Coomassie blue) bands 1.5–2.5 cm behind the albumin band. This group of proteins also stained strongly for carbohydrate.

To determine whether the antivenom activity of *C. atrox* plasma is potent enough to be of potential value in snakebite therapy, we compared its effectiveness with that of antivenin. The plasma was more effective in increasing survival time than any dose of antivenin (Table 2). This is remarkable as whole *C. atrox* plasma was used and, as shown by PAGE, only a small amount of the total plasma protein has antivenom activity. In contrast antivenin is a purified horse serum immunoglobulin preparation. Thus *C. atrox* plasma is a very effective antivenom.

We also found that *C. atrox* plasma activity against *C. atrox* venom was maintained for up to 4 d *in vivo* (Table 2), whereas antivenin rapidly loses its effectiveness (6–12 h) *in vivo* (rabbits).

We found the same antivenom pretreatment effect in mice by determining neutralisation capacity against *C. durisus terrificus* venom as a function of pretreatment time. There was a steady decline in neutralisation capacity during 3 d after a single intraperitoneal injection of antivenom (10 mg protein). The curve is somewhat steeper for *C. durisus terrificus* venom compared with that of *C. atrox*.

In these experiments, treated rabbits and mice showed markedly less tissue damage and haemorrhage than untreated animals. Isolation and characterisation of the antilethal factor(s) in *C. atrox* and *C. adamanteus* plasma may show that it is similar to the antihaemorrhagic factor found in Habu (*Trimeresurus flavoviridis*) venom reported by Omori-Satoh[11].

Even though immunoglobulin antivenin, prepared from sera of horses immunised against venom, is the most effective agent used in the management of snakebite, there are many serious problems associated with its use; for example, allergic reactions and the length of time from the bite to the effective administration of antivenin. Perhaps an antivenom prepared from snake plasma or a synthetic product based on antivenom factor(s) in snake plasma would be useful and safe for both the first aid and medical management of snakebite.

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Erratum

In the article "Calcium activation produces a characteristic response to stretch in both skeletal and cardiac muscle" by R. L. Moss, M. R. Sollins and F. J. Julian (*Nature*, **260**, 619; 1976) there were the following errors: on p. 619, line 16 should have read . . . able to interact with thin filament sites . . . and not as printed. On p. 620, second column, line 27 should have read . . . cium (*p*Ca about 9). Little if any short range elastic response . . . and not as printed.

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reviews

Symbionts or parasites?

Fred Dainton

The Force of Knowledge: The Scientific Dimension of Society. By John Ziman. Pp. ix+374, (Cambridge University: Cambridge and London, April 1976.) £7.50.

FRANCIS BACON firmly believed that natural philosophy could be organised for the benefit of mankind and no doubt good rational men holding power in an authoritarian state might entertain this hope. But both science and society change and so do their interactions. In his book, which arrived on the 350th anniversary of Bacon's death, Professor Ziman traces the changes in this relationship in the period from Bacon to the present day.

It is difficult to encapsulate the author's thesis in a few phrases but he seemed to be saying that knowledge—that is, science—is only useful if it leads to the creation of new knowledge or the solution of social problems or the acquisition by a society of physical power over resources and energy; and for all these purposes it must be communicated between men. But new ideas came from the sifting, re-arranging and assessing sceptically facts and earlier concepts in the minds of individuals. So in the first few chapters we are given glimpses of individual scientists, their styles of work, how their institutions for discourse and intercourse developed from clubs to learned societies and professional associations in response primarily to the increasing need (and difficulty) to communicate.

His characters are skilfully chosen and his vignettes are beautifully drawn: there is the restless, questing intellect of Leonardo; the convergent, introvert Cavendish drawing solitary satisfaction from precise observation and measurement; Ramanujan, the untutored genius; Wegener the inspired advocate of continental drift admitting only the evidence favourable to his belief; the courageous, pugnacious, rational, iconoclastic ideologue J. B. S. Haldane and the brilliant but conservative all-rounder Fermi forced by the social significance of the work he initiated to face up to his social responsibility. All this and much else besides is offered to the reader in felicitous evocative prose accompanied by beautiful illustrations; but I found it curious that such an author should stress the cognitive at the expense of the affective in his subjects. Thus we read nothing of their frequent passion for music and mountains. From the intercommunication of scientists it is a short step to their communication with laymen where, oddly, the roles of the Extra-Mural Departments of Universities, the Open University, of the Literary and Philosophical societies and other mutual improvement movements receive no mention.

Photo: California Institute of Technology

Einstein—personal authority accorded by his own republic of learning

Professor Ziman is at his best in showing us how personal authority whether accorded by the state—for example, as to Lindemann and Oppenheimer—or by their own republic of learning—for example, as to Darwin and Einstein—is in the long run the enemy of progress in science. Healthy science requires the maintenance of a vigorous scepticism in its practitioners, who should not be obedient priests but democratic and critical republicans. But alas scientists are human too and the revolutionary of yesteryear tilting at authority is easily turned by success into the established figure of today resisting the attacks on his ideas, position and influence. After a brief glance at medicine—taken principally, one feels, to illustrate the fundamentally reactionary nature of craft guilds and trade unions exerting powers they have wrested from rather than been willingly awarded by society—the reader moves to stories of industrial innovation (zips, jet engines, nylon, penicillin, float batteries) which the author believes to be "one of the dominant social processes of our time". It may well dominate less in future as such innovation makes further inroads on our resources of energy and raw materials; an aspect which like pollution (*pace* a brief reference on p279) is one of the outcomes of 'science in action' which is completely disregarded.

By now (chapter 9) the reader has been brought to the third quarter of the twentieth century, the scientist is transmogrified from the independent researcher to the more or less willing member of a large team, completely institutionalised, serving the military or civil ends of the state or the commercial ends of a corporation or 'big science'; or, if he is 'academicised', behaving in the ritual research fashion to enliven his teaching and to earn the approval of his senior colleagues which he esteems for its own sake and/or because it aids his promotion. Next follows a look at the transplantation of science from Western to alien soil in Australia, Japan, India and in that hypothetical but so clearly recognisable and authentic newly emerged Republic of Saturnia and the tragi-comic outcome of this dislocation. We proceed to a child's guide to the social sciences where controlled experiments are impossible, all systems are multivariate (and not independent variables at that!) and people still persist in the exercise of 'free will', the greatest 'joker' in the pack. So by way of the rarely felt moral dilemma of scientists, employed in increasing numbers in the war game, we come to the final chapter which is really an essay in the necessity of scepticism of authority. True as this is, it hardly seems to get to the roots of the scientists' social responsibility and it is a pity that Professor Ziman did not allow the current of his fluent prose to carry him to some deeper commitment.

This said we must remind ourselves that the book is the result of his preparing himself, as his conscience dictated he must, "to introduce the theme of the social relations of science and technology" to the average second year university student of natural science. In this aim he has succeeded brilliantly. Such students with 'A' levels in physics and chemistry should be able fully to understand the scientific, technological and engineering content. Moreover, based either on my own recollections of myself 40-odd years ago or on conversations with present-day students I am convinced that the style, topics and sentiments displayed in the book should have a strong appeal to the sense and sensibilities of young adults. They should find it as fascinating as a good novel, replete with characters whose individual and collective attributes are mirrored in many of their seniors and who face issues of great contemporary relevance and importance. I hope that they will put it down with the feeling that their generation has to meet the challenge of how the scientist is to be the critical symbiont of the larger society of which he is a part, helping to shape its purposes rather than becoming either the parasite or the passive helot of a society to whose aims he is indifferent if not hostile.

There is yet another reason why, were I still a professor, I would strongly recommend this book to my students and more particularly to their nonscientific contemporaries. It is simply that the book conveys in a lucid and lively manner the essence of the attitudes of scientists and by marked contrasts of style and matter jolts the reader into new thoughts. Thus it is serious in intent but impish in style, reverant and irreverent by turn, and popular—almost shallow treatment of some matters nevertheless often contain implicit or explicit insights of some depth. They serve to give the book a touch of human frailty seeming to put the author on the same level as the readers and thus encouraging them to think critically rather than be the passive receptors of the dispensed, predigested pabulum of scientific authority proffered to them in the textbooks. But scholars will sigh over inaccuracies and over-simplications or exaggerated phrases, and twice I narrowly escaped apoplexy. No reader will be indifferent and the young will be stimulated as the author wanted them to be. All that remains to be asked for is a paper back at half the price of the hard copy.

Sir Frederick Dainton is chairman of the University Grants Committee of the UK.

Unexpected treasures

The Compacted States of Vitreous Silica. By William Primak (Studies in Radiation Effects in Solids, Vol. 4.) Pp. xv+184. (Gordon and Breach: New York, London and Paris, 1975.) £10.70.

CONSIDERING the terrestrial abundance of silica and the crucial position it holds in several fields of technology, our understanding of its structure and solid-state chemistry is deplorably vague. An excuse can be given—that the solid states of SiO_2 are many and complex and have frustrated many conventional attacks— but the response of science has been lax. Pure scientists, instead of mounting more unconventional attacks, can be accused of generally turning away from the problems and letting the technologist flounder. A short list of technological uses of silica includes transmitting and reflecting optics, ceramics, piezoelectric devices, crucibles, passivating and insulating films; some unusual thin-film cantilever structures are now being used for displays and other purposes. In many of these applications, vitreous silica is used and yet, because conventional crystallography cannot work on such amorphous structures, ignorance of this state of the material is the greatest.

Primak's book-length review article presents a highly individual view of the structure of this amorphous material, based on physical measurements which can certainly be classed as unconventional but were superbly adapted to the problem. Compaction is an effect which occurs only in non-crystalline solid networks (perhaps even only in oxide networks but Primak omits to tell us if any non-oxide glasses will compact); it is produced by pressure-cycling, high temperature, shock waves or radiation and can so alter the vitreous silica network that density is permanently reduced as much as 15% for pressure, and 3% for the others. Alkali silicate glasses will compact even more. This behaviour is so foreign to our concepts of immutable lattice constants that it is scientifically important; technological uses are, of course, also conceivable. Primak's own work, mainly on radiation-induced effects, is a classic of well-performed experimental study, augmented by careful but not hidebound interpretations. His hypothesis for the compaction was startling in that it went clean against the instinctive feeling of other workers that an increase in density implied an increase in order (fused silica is less dense than quartz).

He supplies ample evidence that silica can reduce its volume by the formation of a 'kink' in the large (say twelve-atom) rings of the silica network so that a 'void space' which was previously empty is newly occupied and order thereby decreased. But why should the kink be retained? The wider, new idea comes in the proposal that the silica network is in "a microscopic state of balanced stress" and that an excitation, producing one kink, causes the whole local structure to rearrange, perhaps in the form of a "network segment" which re-configures its quota of non-bridging oxygen atoms. Although Primak does not elaborate the model with any theoretical solid-state physics, it is still a very meaty and useful hypothesis. The book is a pursuit of its proof through every scrap of evidence available (there are over 350 references). Although the book was conceived nine years ago and largely finished six years ago, the central hypothesis and the suggestions for further investigations remain valid. Appendices embrace new research up to 1973.

Only occasionally does the relaxed personal style lead to vagueness; for example, the author never tells us the best estimate for the Si–O–Si bond angles and lengths in the seven main phases of silica, and this sort of thing does detract from the introductory value. Later chapters are packed with intense and creative reasoning. Parochialism sometimes shows up here—for example in the lack of use which Primak has made of the electronic–optical disciplines of solid-state physics—for example, he does not explain the relevance of infrared spectra, or more than quote a few measurements. His only definite error in the electronic field is to say that "vitreous silica has no proper band structure", a common but unfortunate misconception, disproved by Di Stefano and others; the field of colour centres is dismissed as a "morass" and, although work on thin silica films is mentioned in passing, he has failed to note a valiant attempt made in 1969 by H. L. Hughes to explain γ-ray-induced surface-state generation in such films, using a compaction model correlating his and Primak's data. One feels that great benefit would have been bestowed on the world if the author had wandered a bit further in these fields.

The book does give people interested in silica a chance to revel in a refreshing minor tributary of knowledge which may yet do great things for the mainstream. By his pleasant but creative style the author indicates some of the unexplored creeks which could yield unexpected treasures from the most abundant material in the earth's crust.

Andrew Holmes-Siedle

Aphids

Aphids. (Invertebrate Series.) By Roger Blackman. Pp. 175. (Ginn: London and Aylesbury, 1975.) £3.00.

THE main reason why aphids have not attracted the attention they merit is the lack of simple accounts of their biology and taxonomy. Roger Blackman in 175 pages presents such a comprehensive, highly readable and extremely well illustrated account of aphids. The book's main asset is Chapter 10, 'Aphids in Britain'. This chapter makes available to amateur entomologists, for the first time, a workable key in English to the more common genera of aphids in Britain. The excellent colour plates by Hilary Burn, which illustrate this chapter, serve to bring some of the aphids to life. Unfortunately the colour photographs in plate 1 are so badly reproduced that they are useless; an additional colour plate would have been preferable.

The other 10 chapters deal with the structure and physiology of aphids, natural enemies, relationship with ants, population dynamics and techniques of study. This last chapter is a useful source of ideas for student projects.

In future editions Fig. 3 could be omitted, or revised, as it confuses rather than clarifies the discussion of an aphid's powers of multiplication; and Fig. 33 modified, as not all host-alternating aphids can produce and mature sexual morphs on their secondary hosts. The complex terminology of the various morphs of aphids has deterred many biologists from studying aphids, and it is therefore surprising that Dr Blackman did not use the simpler terminology proposed by Hille Ris Lambers (*A. Rev. Ent.*, **11**, 47–78, 1966). Unqualified statements such as aphids produce large numbers of offspring to counteract the losses they incur (p34), and that crowding is self induced (p78) unfortunately support Janzen's (*Science*, **182**, 1125–1126, 1973) contention that aphid biology is in need of a solid dose of evolutionary biology. Apart from these criticisms this book can be warmly recommended as an excellent introduction to a fascinating group of insects.

A. F. G. Dixon

Controversial texts

Techniques of Applied Quantum Mechanics. By J. P. Killingbeck. Pp. 224. (Butterworth: London and Boston, Massachusetts, November 1975.) £9.

OF the five chapters in this book two are directly relevant to the title—chapter four on "Perturbation and Variational Methods" and chapter five on "Group Theory", both topics in which the author has had practical experience. It is a pity that chapter four did not begin at the beginning—clearly a reader is supposed to have already read a standard book on quantum mechanics including the section on perturbation theory. The book would have been better if these chapters had been preceded by a conventional description of the principles of quantum mechanics. Instead we have a chapter called "Review of Basic Principles", which makes derogatory remarks on other textbooks, gives instructions to teachers of quantum mechanics and tells us about dyadics, but fails to review the basic principles. It is too nebulous in intent to be useful and is inconsistent in what is required of the reader—for example, if he needs to be told the meaning of operators commuting he should not be expected to be familiar with Dirac bra/ket notation. We then have two chapters on "Modern Mathematics" and "Operator Theory". In fact the mathematics of the second chapter is not in any sense modern. As a summary of some, mainly algebraic, topics these two chapters are adequate and probably useful. They are, however, essentially a list of definitions and theorems. Little is actually done with the concepts discussed and there are no proofs. It is hard to see how any student can really learn a subject from this type of treatment.

When the author is writing about "Applied Quantum Mechanics" he writes well and it should be said that the relevant two chapters constitute more than half of the book. In other places he is less convincing and one detects a certain 'propaganda' aspect to to the book, inappropriate in a textbook. On p10: "One of the basic problems is that of communication between the different types of scientist working on the subject; many a physicist has tried to follow up some interesting point which he suspects may have a practical application, only to give up in frustration at the large number of unfamiliar theorems and erudite subtleties which are taken for granted by the average mathematician's writings on quantum mechanics". Presumably it is "lack of communication" not "communication" that is alleged to be a problem. But is it a problem? What is the evidence?

On p38 we are told "The embedding of the real numbers in the complex numbers which is involved in this calculation is usually not commented on at all in texts concerned with quantum mechanics". Presumably these textbooks, contrary to the one under review, also omit to tell us that ±i is "more exactly 0 ± li". Maybe their authors know something! **E. J. Squires**

More to coral reefs . . .

Biology and Geology of Coral Reefs. Vol. 3 (Biology 2). Edited by O. A. Jones and R. Endean. Pp. xxi+435. (Academic: New York, San Francisco and London, 1976.) $49.00; £25.00.

FOR the second biological volume of this composite work on coral reefs, the editors have assembled a highly competent team of collaborators. There are chapters dealing with the more important groups of associated organisms and others covering aspects of the ecology of both reefs and coral cays. (For a review of volumes 1 and 2, see *Nature*, **248**, 809, 1974.)

From the unique wealth of his long experience, F. R. Fosberg discusses coral island vegetation which, in its limited but widely distributed species, gives, as he concludes, an insight into the nature and complexity of vegetation generally, while itself forming the basis of the rightly famed beauty of coral islands.

A. J. Bruce reviews our present knowledge (to which he has contributed so extensively) of the numerous shrimps and prawns around coral reefs. Aspects of the ecology of coral reef fishes are similarly discussed by B. Goldman and Frank Talbot, again on the basis of much personal experience on One Tree Island in the Capricorn Group. A further chapter deals with the natural toxicity of certain coral reef fishes.

The highly vexed problem of ciguatera is the province here of its major investigator, A. H. Banner. This disease, which may unpredictably follow human consumption of coral reef fishes in the Indo-Pacific and the Caribbean, forms a major hazard in the developments of fisheries. We know the nature of the toxin but not the conditions controlling its appearance.

The factors responsible for the destruction and recovery of reefs are very suitably reviewed by R. Endean; there are further chapters on echinoderms, on intimate associates of reef corals and on birds and on turtles, the last from Robert Bustard who has contributed so much to our knowledge of those around the Great Barrier Reef.

Altogether this is an exceptionally valuable book to be studied and, if possible, possessed by the growing numbers of those fascinated by the innumerable problems presented by a living coral reef.

C. M. Yonge

obituary

Robert Franklin Mehl, Professor-Emeritus of Metallurgy at Carnegie–Mellon University, who died in Pittsburgh on January 29 at the age of 77 was undoubtedly one of the greatest academic metallurgists of the American scene. After studying at Franklin and Marshall College, Princeton and Harvard Universities, he went to the Naval Research Laboratory in 1927 as Director of the newly formed physical metallurgy laboratory. In 1932, after a brief period in industry, he became the first Director of the Metals Research Laboratory at the then Carnegie Institute of Technology, and launched on what was to be his greatest achievement. He collected around him an extremely talented group of physicists, chemists and metallurgists who in the next decade were to make outstanding contributions to physical metallurgy, particularly in the fields of diffusion, phase transformations and precipitation hardening. But perhaps Mehl's greatest contributions were towards a basic understanding of the various reactions involved in the heat treatment of steels, in particular the formation of ferrite from austenite and the pearlite and bainite reactions which he and his colleagues analysed so thoroughly that they elucidated many of the principles from which the physical metallurgy of steels has developed.

In 1935 Mehl also became Chairman of the Department of Metallurgy, instituting scientifically based courses of high standard which in a few years made Carnegie Tech. a premier place for the study of metallurgy which had a large impact not only on the local steel industry but also throughout the United States. During World War II his laboratory was thus well placed to carry out work which greatly aided the production of guns and armour, though Mehl was often frustrated by reactionary attitudes in industry towards scientific endeavour!

After the war, he continued to stimulate the activities of the Metals Research Laboratory over an extremely wide range until 1960. Possessed of a very strong personality, he wielded his influence far and wide; inevitably his scientific contributions were now made with a broad brush rather than a rapier; however, the rapier was always ready at hand to deal decisively with anyone who might venture to take an opposite viewpoint. Numerous names of scientists in the MRL during the period 1945–60 now adorn the faculties of many well known departments of metallurgy and leading industrial laboratories, a constant reminder of the high standards on which Mehl insisted.

He became a familiar figure in Europe between 1960 and 1966 when he was European consultant to the US Steel Corporation. The combined role of ambassador and interpreter of the European metallurgical scene suited Mehl at this stage of his life, when he was already one of the grand old men of metallurgy; he returned, however, to the USA in 1966 to become visiting professor at the University of Delaware and later at Syracuse University. Latterly, retired in Pittsburgh very close to his beloved University, he suffered severe ill-health, but set a fine example by his great courage, and by maintaining an active interest in his subject to the last. A paper he wrote in 1975 for the Annual Review of Materials Science is an excellent summary of his scientific career and is spiced with his own unmistakeable philosophy. It is indeed a fitting epitaph.

R. W. K. Honeycombe

Appointments

Dr Mart de Groot as Director of the Armagh Observatory.

Dr Thomas Anthony Connors as Director of the MRC Toxicology Unit.

Dr Peter Cardwell Elmes as Director of the MRC Pneumoconiosis Unit.

Dr R. R. Dils as Professor of Physiology and Biochemistry at the University of Reading.

Dr A. F. G. Wyatt as Professor of Physics at the University of Exeter.

Announcements

May 27–28, **Biology of Chemical Carcinogenesis,** London (The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG).

June 3, **Contributions of Protozoa to Genetical Knowledge,** London (The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG).

June 17–18, **Quantitative Mass Spectrometry in Life Sciences,** Ghent (Prof A. De Leenheer, Laboratoria voor Medische Biochemie en voor Klinische Analyse, Academisch Ziekenhuis, Rijksuniversiteit Ghent, 135 De Pintelaan, B-9000 Ghent, Belgium).

June 22, **Reclamation and Recycling of Metals,** London (The Meetings Secretary, The Institution of Metallurgists, Northway House, Whetstone, London N20 9LW).

June 26, **Educational Technology for the Handicapped Child,** Birmingham (P. R. Davie, Prospect Hall, Selly Oak Colleges, Birmingham B29 6LE).

October 16–20, **Molecular Basis of Host–Virus Interaction,** Varanasi, India (Dr M. Chakravorty, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, India).

October 18–21, **Annual Meeting of the Association of Official Analytical Chemists,** Washington DC (L. G. Ensminger, Executive Secretary AOAC, Box 540, Benjamin Franklin Station, Washington, DC 20044).

November 17–19, **ACTH and Related Peptides: Regulation, Action and Structure,** New York (Conference Department, The New York Academy of Sciences, 2 East 63rd Street, New York, New York 10021).

Erratum

In last week's **Appointments** section the new Professor of Botany at Royal Holloway College was incorrectly given as Dr J. D. Hodge. It should have been Dr J. D. Dodge.

Reports and publications

Great Britain

Family Planning in Five Continents: Africa/America/Asia/Europe/Oceania. Pp. 28. (London: International Planned Parenthood Federation, 18/20 Lower Regent Street, W1, 1975.) 65p; $1.45 plus postage. [52

Bulletin of the British Museum (Natural History). Geology. Vol. 27, No. 1: A Reappraisal of *Prophaethon shrubsolei* Andrews (Aves). By C. J. O. Harrison and C. A. Walker. Pp. 1–30 + 3 plates. (London: British Museum (Natural History), 1976.) £2.85. [92

Mineral Resources Consultative Committee. Mineral Dossier No. 15: Mica. Compiled by R. N. Crockett. Pp. v + 22. (London: HMSO, 1975.) 85p net. [92

Department of the Environment. Register of Research 1975. Part 4: Environmental Pollution. Pp. v + 258. (London Headquarters Library, Department of the Environment, 2 Marsham Street, SW1, 1975.) [102

Ministry of Agriculture, Fisheries and Food. Agricultural Chemicals Approval Scheme: Insecticides/Fungicides/Herbicides. 1976 List of Approved Products and their Uses for Farmers and Growers. Pp. 190. (Hatching Green, Harpenden: Agricultural Chemicals Approval Organization, Ministry of Agriculture, Fisheries and Food, Plant Pathology Laboratory, 1975.) [112

nature

May 27, 1976

PhD in evolution

EIGHT months ago the Science Research Council (SRC) produced a report on postgraduate education which by the standards which generally prevail in the academic world was decidedly radical. The working party, headed by SRC chairman Sir Sam Edwards, proposed amongst other things that greater emphasis be placed on graduate students taking a wide range of courses, particularly in their first year. A high proportion of broadly based compulsory course work leading to an examination for a Master's degree or equivalent qualification was suggested. The option of continuing for a further two years, the report went on, should only be open to students who had passed the Master's course and demonstrated real aptitude for research. The example of MIT was clearly well to the fore in the working party's deliberations.

The background to this needs little rehearsing. In the 1960s PhDs were being signed up with enthusiasm, not least by the new universities which then proceeded to generate even more PhDs of their own. These days universities no longer recruit, emigration is less of an option, the civil service does not grow in size, so PhDs find themselves applying for a job, somewhat reluctantly maybe, to those same industries to which they might have applied three years earlier. But industry's view of a PhD is by no means one of universal approbation—indeed, those in industry who think that PhDs are of little use to their needs have been getting much of the attention in the last year or two. Small wonder then that SRC decided that some spring cleaning was necessary of the ideas surrounding the PhD.

In the past two months a form of participatory democracy has been in operation. SRC had already received many written submissions on its report, but it decided to take to the road as well so that the report could be discussed more informally in regional gatherings of university science staff and industrialists. That exercise, which took the SRC to Cardiff, Birmingham, Glasgow, Leeds and London, is complete; now the SRC must try to see whether anything like a coherent policy can emerge from the consultative process. The general conclusions that can be drawn seem to run as follows:

- Industry does not speak with anything like a unified voice in its views about the relevance of the PhD. Even within the same company it is possible to find, say, research directors and personnel managers who would differ in their assessment of the value of the degree.
- Whilst most academics support, or at least don't oppose, the idea of more taught courses, the idea that these should be compulsory has had a very mixed reception.
- The idea that the courses should be concentrated in the first year of postgraduate education has come in for some criticism.
- The nature of the assessment at the end of the first year has caused some alarm, not surprisingly amongst students who fear another year of being tyrannised by examinations.
- Many people have expressed doubts about the feasibility of doing a good research project for a PhD in two years rather than three.

Some of these points will undoubtedly result in a softening of the SRC's approach; more than once the council was urged to encourage evolution rather than impose revolution, and such changes as spring from the exercise are likely to emerge slowly over the next decade. For it is not at all clear that the SRC should use the muscle that it has, in the way of discretion over grant money, to force the pace. Maybe the very fact of the report and the chance to air views will have generated enough momentum.

The one thing that the SRC should not soften on, however, is its conviction that there is not a "correct" duration of three years of research for the PhD. A fairly common view amongst academics was that a period of less than three years in the laboratory is inadequate for students to make a research contribution. If the student is being trained to write papers or know more about some particular subject than anyone else in the world, this may have some element of truth in it. But industry does not usually hire its PhDs for either of these reasons; it hires them for their potential to work in a broadly based team. And the PhD who stays in the academic world is going to have plenty of time to develop the necessary expertise later.

Moreover, it is a poor comment on the quality of postgraduate course work if it doesn't make a significant contribution to helping students to be more effective researchers, by pointing them in the direction of key literature, by exposing them to the ethos of research and by allowing them the luxury of sampling different staff members before having to make a final choice. A year of coursework could easily be worth two of research. ☐

US takes up the issues . . .

The nuclear exporters meet again soon. **Colin Norman** reports on the debate now taking place in the United States

NEXT month, representatives of present and potential nuclear exporting countries are scheduled to meet in London for a second round of secret talks aimed at curbing the proliferation of nuclear weapons. With international trade in nuclear goods now estimated to be well in excess of $1,000 million a year and growing rapidly, and with several particularly risky transactions in the works, the talks are considered by many to be critical for the future stability of the world.

They will take place against a background of growing demands in the United States for extraordinary measures to prevent potentially dangerous nuclear transactions from taking place. Though some of the strongest demands are coming from Capitol Hill, the issue of nuclear proliferation has even been raised in the Presidential election campaign, and some Administration officials are also taking an increasingly hard line in urging the adoption of strict international safeguards to prevent the diversion of peaceful nuclear technology to weapons production.

Two recent developments are, however, likely to be particularly influential in next month's talks. On May 14, the Senate Foreign Relations Committee approved a foreign aid bill containing a little-noticed provision which would cut off all US aid to countries buying or selling uranium enrichment or nuclear reprocessing plants without adequate safeguards. And, in the same week, the Senate Government Operations Committee approved a bill calling on the President to negotiate a "binding agreement" with other nuclear exporting countries to prohibit the sale of enrichment or reprocessing technology to individual countries which do not already possess nuclear weapons. The bill also specifies, that the agreement should prohibit the sale of any nuclear facility—including a power reactor—to a country which refuses to place *all* its nuclear activities under international safeguards and inspection.

Moreover, Senator Abraham Ribicoff, the chairman of the Government Operations Committee, has on several occasions called for strict sanctions against West Germany and France if those countries go ahead with plans to sell reprocessing plants to Brazil and Pakistan respectively.

Thus, with Congress breathing heavily down its neck, the Ford Administration is likely to take a tough line in next month's talks. Because some participants in the talks have insisted on strict secrecy—France is said to have threatened to pull out of the first round if the discussions were made public—Administration officials are reluctant to discuss that matter in detail. Nevertheless, it is clear that there is likely to be continuing friction between the United States on the one hand, and France and West Germany on the other.

The first round of the talks, which included representatives from the United States, the USSR, Britain, Canada, France, West Germany and Japan, ended in January with general agreement on principles for international safeguards. According to assorted reports and Administration sources, the agreement would allow nuclear technology to be sold only to those countries which promise not to use it to produce explosives, and it would provide for the adoption of common standards to guard against theft or clandestine diversion of nuclear materials.

The agreement fell far short, however, of the strict controls urged by the United States. The Secretary of State, Henry Kissinger, for example, said in Congressional testimony on March 9 that the talks had simply ended in a "general understanding about restraint", and he suggested that the United States would press for "something more binding" in the next round. The Ford Administration is particularly concerned that the agreement is weak in dealing with the critical issue of the sale of uranium enrichment and fuel reprocessing plants.

Because enrichment plants are capable of producing weapons-grade uranium, and reprocessing plants separate plutonium—another potential bomb ingredient—from spent reactor fuel, they are the key links in a weapons fabrication process. By themselves, reactors present a smaller proliferation hazard since neither the uranium fuel nor the reactor wastes can be used directly to make explosives. Thus reactor sales to non-nuclear weapons countries are usually made on condition that the fuel going into and coming out of the reactor is closely monitored, and that it is not enriched or reprocessed by the purchaser.

The International Atomic Energy Agency (IAEA), the Vienna-based body established in 1957, is responsible for policing most nuclear transactions to ensure that no diversion of weapons-grade material takes place. IAEA keeps records of fuel passing through reactors under its supervision, and it also has a small corps of inspectors authorised to make spot checks at individual installations. The Nuclear Non-Proliferation Treaty (NPT), which came into force in 1970, binds all signatories to place future nuclear transactions under IAEA supervision.

A number of recent events have, however, shattered hopes that such a loose system of controls can be completely relied upon to prevent the insidious spread of nuclear weapons around the world. The most significant event by far was the detonation in 1974 of an explosive device by India. Using plutonium reprocessed from spent fuel from a Canadian-supplied reactor, India abruptly ended the nuclear hegemony of the United States, the USSR, Britain, France and China. Though Indian officials insisted that they would only use nuclear explosives for peaceful purposes, that piece of semantic juggling does not disguise the fact that India is now a nuclear power.

The other chief unsettling events were the negotiation last year of an agreement between West Germany and Brazil for the sale of an entire nuclear system, including enrichment and reprocessing facilities, followed by a deal between France and Pakistan involving the sale of a reprocessing facility. A third transaction, the sale of a French reprocessing plant to South Korea, was scotched earlier this year when South Korea pulled out under heavy pressure from the United States. (In a press briefing in Washington last

Dr Fred Iklé, Director of the US Arms Control and Disarmament Agency week, however, the French President, Valery Giscard D'Estaing, said that he had personally vetoed the sale.) West Germany, in addition, is widely believed to be negotiating to supply reactors, and possibly a reprocessing plant, to Iran.

Those deals mark a significant qualitative shift in international nuclear trade, since they would transfer directly the means for producing weapons-grade materials. What makes them particularly worrying is the fact that none of the recipients, except for Iran, is a party to the NPT. They have all been bitterly opposed by the United States, and US officials from Dr Kissinger down tried in vain to dissuade France and Germany from going through with them.

Aside from sales of enrichment and reprocessing technology, there has also been considerable doubt expressed recently about the adequacy of present safeguards on reactor sales. For one thing, many reactors have already been sold without IAEA safeguards—the Canadian sale to India provides a particularly vivid example, and similarly the sale by France of a small research reactor to Israel in the early 1960s is widely believed to have provided Israel with the means to produce weapons. According to a recent CIA analysis, Israel has taken up that option and now possesses between 12 and 20 plutonium bombs.

And the IAEA safeguards themselves do not provide an insurmountable barrier against diversion of fissile material. In a speech on May 13, Dr Fred Iklé, Director of the Arms Control and Disarmament Agency (ACDA) and one of the more outspoken Administration officials on nuclear proliferation, warned that the IAEA is understaffed, relies chiefly on information supplied by the country under safeguards, has no power to impose sanctions against violators and cannot investigate unsafeguarded plants. IAEA safeguards, Iklé said, provide "a burglar alarm, but not a lock", and it is a "fallacy" to believe that we don't have to worry about facilities under IAEA safeguards.

The United States itself, moreover, is not entirely blameless. In 1974, then President Nixon promised to sell nuclear reactors to Israel and Egypt, two antagonists who have not ratified the NPT. (Negotiations concerning those reactors are not yet complete.) And last week it became known that the General Electric Corporation has applied for a licence to sell two 1,000 MW reactors to South Africa, together with 1.4 million pounds of slightly enriched fuel. According to Administration sources, that deal is likely to be officially approved, even though South Africa has not signed the NPT and is believed to be interested in joining the nuclear weapons club.

That is the background against which the talks between the nuclear exporting nations will resume next month. Aside from the seven original participants, they are expected to include representatives from the Netherlands, Sweden, Belgium, East Germany, Italy and Poland. The critical issue is again expected to be the sale of enrichment and reprocessing technology, with the United States arguing against deals which would place such plants in the hands of individual purchasers, whether or not they are placed under IAEA safeguards.

The chief American argument is simply that reprocessing is not necessary at this time, and there is no economic need to sell either enrichment or reprocessing plants. Dr Iklé, for example, noted in his May 13 speech that separating plutonium from spent fuel and recycling it "could replace at most about one third of the fuel required, and far less in a rapidly growing nuclear power system. Hence, recycling would not bring independence from imported fuel". He added: "Before we plunge into a plutonium fuel economy, let us look very closely at the risks and our ability to control them . . . spreading plutonium should be avoided if possible, and with the current generation of reactors it can be avoided at no economic cost".

If it proves impossible to curb the desire for reprocessing plants, the Administration will probably continue to urge that instead of selling such plants to individual countries, nuclear exporters should consider placing such technology under multinational control. Iklé noted that the United States is now studying the feasibility of "multinational fuel centres for storage of fuel, waste management, and other services when needed".

The United States' arguments are, however, likely to fall foul of the desire,

Passage to India?

THE Nuclear Regulatory Commission (NRC) announced last week that it will hold public hearings to decide whether or not the United States should supply slightly enriched uranium to India, for the Tarapur Atomic Reactor located near Bombay. The hearings, the first ever to be held on a nuclear export licence application, graphically underline the fact that because the United States is the world's major supplier of reactor fuel, it is in a strong position to force many countries to accept strong safeguards against the diversion of peaceful nuclear technology to weapons production.

The hearings, set for June 2, will be held in response to a petition from the Natural Resources Defense Council, the Sierra Club and the Union of Concerned Scientists. The United States has supplied India with fuel for that reactor in the past, but the petitioners are essentially suggesting that NRC should use the application to supply another 40,000 pounds of uranium as a way to force India to accept extra safeguards.

The Tarapur reactor and its fuel supplies are all under the supervision of the International Atomic Energy Agency (IAEA), and those facilities were not the source of plutonium for India's nuclear explosives.

But the petitioners have pointed out that India hasn't signed the non-proliferation treaty, that a clash between India and one of its neighbours might disrupt present safeguards at the plant, that the United States has not required India to place all its other nuclear facilities under international safeguards, and that the United States has not required India to accept bilateral safeguards in addition to the IAEA controls. The implication is that the United States should threaten to shut off fuel supplies to India unless it accepts those additional safeguards. The concept could clearly be applied to other countries.

At present, only the United States and the Soviet Union export enriched uranium fuel, and their dominance of the fuel export market is expected to last at least until the mid-1980s. Some observers have therefore raised the possibility of a joint US–USSR agreement that future fuel supplies should carry strict safeguards agreements, in addition to IAEA controls. Senator Abraham Ribicoff, for example, has even suggested that the United States should refuse to supply nuclear fuel to West Germany and France if those countries persist in selling enrichment or reprocessing plants. But Henry Kissinger has forcefully ruled out such "blackmail", suggesting that a pact with the Soviet Union against America's NATO allies would have "the gravest foreign policy consequences".

As for the proposed fuel shipment to India, the NRC has announced that it will decide whether to issue the licence before the end of June, and it has suggested that it might even act on the matter before the conclusion of the hearings "if it finds a need for greater expedition". The hearings would, however, carry on with an examination of the broad policy issues involved in United States' fuel exports.

particularly on the part of France and West Germany, to earn maximum export dollars from nuclear trade, and of deep European suspicions of American motives. According to some observers, there is concern in Europe that American attempts to prevent the German–Brazil deal, in particular, were chiefly designed to protect the United States' commercial interests there.

Such suspicions were given a blast of fertiliser last year by an incident which occurred just as the United States was applying pressure on West Germany not to conclude its fuel technology agreement with Brazil. According to published accounts, a representative of the Bechtel Power Corporation, a major US nuclear manufacturer, met in March last year with Brazilian officials to discuss the possibility of building fuel facilities there, leaving the impression that the US government would sanction such a deal. Though the incident was probably simply a product of poor communications between industry and government in the United States, it clearly left a sour impression in Europe. Moreover, it should be noted that while Dr Iklé has been arguing that there is no economic incentive for recycling plutonium at present, the US nuclear industry has been urging the government to allow plutonium recycling in the United States.

If the provision in the foreign aid bill survives the rest of the Congressional mill intact, however, it would greatly stiffen the administration's policies in trying to dissuade the sale of enrichment and reprocessing technology. Proposed by Senator Stuart Symington, it would cut off nearly all US aid to the buyers and sellers of such technology, unless they have agreed to "place all such equipment, materials, and technology, upon delivery, under multilateral auspices and management when available", and unless the recipient agrees to place all its nuclear facilities under IAEA safeguards. The latter provision is intended to prevent the recipient from either duplicating the reprocessing or enrichment technology, or separating plutonium from an unsafeguarded reactor in the transferred reprocessing plant. The amendment is not, however, included in the House version of the bill, and its prospects are uncertain.

As for safeguards on the sale of power reactors, the Administration's policy is that controls should be applied uniformly among the supplying countries rather than imposed unilaterally, the argument being that if the United States insists on excessively strict safeguards on its own sales, potential purchasers will simply look elsewhere. Key issues in next month's talks are likely to include ways to bring presently unsafeguarded reactors and facilities into the IAEA safeguards system, methods to ensure that promises not to use imported nuclear technology for weapons production are made binding, and strengthening of the IAEA safeguards and inspection system.

Aside from the suppliers' conference, Mr Jimmy Carter, the leading candidate to be the Democratic Party's Presidential nominee, has called for a UN-sponsored World Energy Conference to discuss worldwide energy problems and alternatives to nuclear power. Calling nuclear proliferation a "fearsome prospect", Carter also urged a ban on sales of reprocessing and enrichment technology, and he called for a pact among purchasers of nuclear technology to buy only from suppliers who require proper safeguards. "The hour is too late for business as usual", he said. So far, nobody else has talked about those issues in the campaign.

The outcome of the exporters' conference is clearly going to be of immense importance. As Denis Hayes, a researcher with the Worldwatch Institute noted in a recent study on nuclear power, if a few more nations acquire nuclear weapons, there will come a point at which the "dam will break and the world will go nuclear". And, in Congressional testimony earlier this year, David Lilienthal, the first chairman of the US Atomic Energy Commission called for an immediate, unilateral halt to all US nuclear exports because of the dangers of proliferation. "I'm glad I'm not a young man", he said, and "I'm sorry for my grandchildren". □

... Canada makes up its mind

Canada announced last week that further nuclear cooperation with India was impossible.

David Spurgeon in Ottawa gives the background

THE Canadian government is just emerging from some serious soul-searching about the moral, political and economic questions involved in nuclear assistance and reactor sales abroad — particularly to developing countries. In the Canadian House of Commons last week, where the subject has been under debate for some time, the External Affairs Minister, Mr Allan MacEachen, announced that the government had decided that it will not resume supplies to India of nuclear equipment and technology. The two countries have failed to agree on safeguards against the use of the materials supplied by Canada for nuclear explosions.

The decision marks another step in the development of Canadian nuclear policy. The whole subject—which an MP described in one session as "perhaps the most important ever raised on an opposition day"—originally arose in the House as a result of government negotiations to resume nuclear assistance to India. In March these negotiations were conducted with India in New Delhi by Ivan Head, foreign policy adviser to the Prime Minister, Pierre Trudeau, and Michel Dupuy, an assistant under-secretary in the external affairs department.

This had followed the suspension of assistance after India's explosion of what it called a peaceful nuclear device in May, 1974. The device used plutonium from the Canadian-design CIRUS research reactor, and the suspension had been ordered because, in the words of MacEachen, "the carrying out of that explosion was in clear violation of the understanding that had been reached between Canada and India".

The latest news probably means that the efforts to reach international understandings on nuclear exports have suffered something of a setback. But the Canadian government has meanwhile been pursuing its chosen path in negotiations with other countries. Canada hopes to obtain an agreement with Pakistan not to use plutonium from a Canadian-designed nuclear power plant, and in January concluded agreements to build 600 megawatt power reactors in Argentina and South Korea. An Atomic Energy of Canada Ltd (AECL) spokesman has said there is the prospect of building second units in Argentina and South Korea in future, and preliminary discussions have been undertaken with other countries, including Mexico. Canada is also involved in licensing negotiations with Italy and Romania. Altogether, expected exports of CANDU reactors between 1974 and 1983 have been estimated by the federal department of industry, trade and commerce to amount to $3,000 million.

The debate over the propriety of Canada's selling nuclear reactors and technology when world powers are concerned about the proliferation of nuclear weapons has gone beyond Parliament. The Prime Minister has received delegations from the Canadian Council of Churches, the United Church of Canada, and the Quakers, and he has been urged to call a halt to the sales. The irony is that the CANDU nuclear reactor has turned out to be one of the most successful in the world at a time when confidence in reactor reliability is waning throughout the world and construction starts are falling off. Canada went it alone with its design (the CANDU is moderated by heavy water and fuelled with natural uranium), and finally appears able, as a result of worldwide interest, to recoup some of its huge investment made over the years.

Critics of the government's policy have expressed fears that CANDU reactors will be used to recover plutonium (produced as a by-product) for the making of nuclear weapons. In the eyes of T. C. Douglas, the former leader of the New Democratic Party, for example, "Canada has greater special responsibility than any other country"; the CANDU reactor, he points out, produces twice as much plutonium as light-water reactors, and spent-fuel rods containing plutonium can be removed daily without shutting down the reactor. On the other hand, Frank Maine, a Liberal MP with a PhD in organic chemistry who is one of the few members of the House with a background in science, has claimed that there are "technical reasons why the CANDU does not contribute to the proliferation of nuclear weapons."

The argument was put in these terms. Since the CANDU is fuelled with natural uranium it converts a lot of its primary fuel component, Uranium 238, into Plutonium 239 and Plutonium 240. Plutonium 239 is used primarily to make bombs, but Plutonium 240 is not very useful for bomb-making. The operating conditions for bomb-making and electricity-generation, however, are contradictory in a CANDU reactor: the longer the fuel is left in the reactor, the more plutonium 240 is made; the shorter the time, the more plutonium 239; but the longer the reactor is left running without changing fuel, the more economic it is. To make plutonium for a bomb, the fuel rods must be left in the reactor for perhaps 600 megawatt days per tonne; but if economic electricity production is wanted, the rods must be left in for 6,000 megawatt days.

Thus, said Dr Maine, the CANDU system is "a total design":

> It is designed to be fed the fuel continuously, and the feeding of the reactor is such that the uranium will be irradiated to the 6,000 megawatt days per tonne level.
>
> If you change it by a factor of ten, either you have to redesign your system completely . . . or else you will have to shut the system down to take out the fuel . . . if you want to generate electricity you use the CANDU system, but if you want to make bombs you do not use the CANDU system.

Dr Maine's technical arguments had little effect on the debates. What bothered many of the opposition members was what they considered a secretive attitude on the part of the government concerning its negotiations with India. Mr Douglas complained that when newspaper reports first appeared about the Delhi delegation and inquiries were made, the Secretary of State for External Affairs "said that the delegation was in India and until it returned he was not in a position to say what agreement it might be discussing". When the delegation did return, "he said the matter was before Cabinet and he could not discuss it until a decision had been made."

Another aspect that bothered critics was that they felt they did not know exactly what was decided at the meetings in London of the "Group of Seven". The Government's position has been that it cannot in good conscience withhold from developing countries the technology they need to help supply their power requirements. Mr MacEachen has also claimed that Canada has had considerable influence in upgrading nuclear standards among the suppliers, and that if Canada withdrew from the export market it would lose this influence. There would also be domestic consequences, such as a radical dislocation of the uranium mining industry. "If we want to succeed in the task of ensuring non-proliferation, we must cast the net wider", Mr MacEachen told the Commons. "We must consider the causes of international tension and do something about the disparities that exist in the world. We must do something to bring about a better and more equitable international economic system."

In addition to the CIRUS research reactor from which India obtained the plutonium for its nuclear explosive device, Canada has helped to build two reactors known as RAPP-I and RAPP-II. Completion of the second has been held up by Canada's decision not to

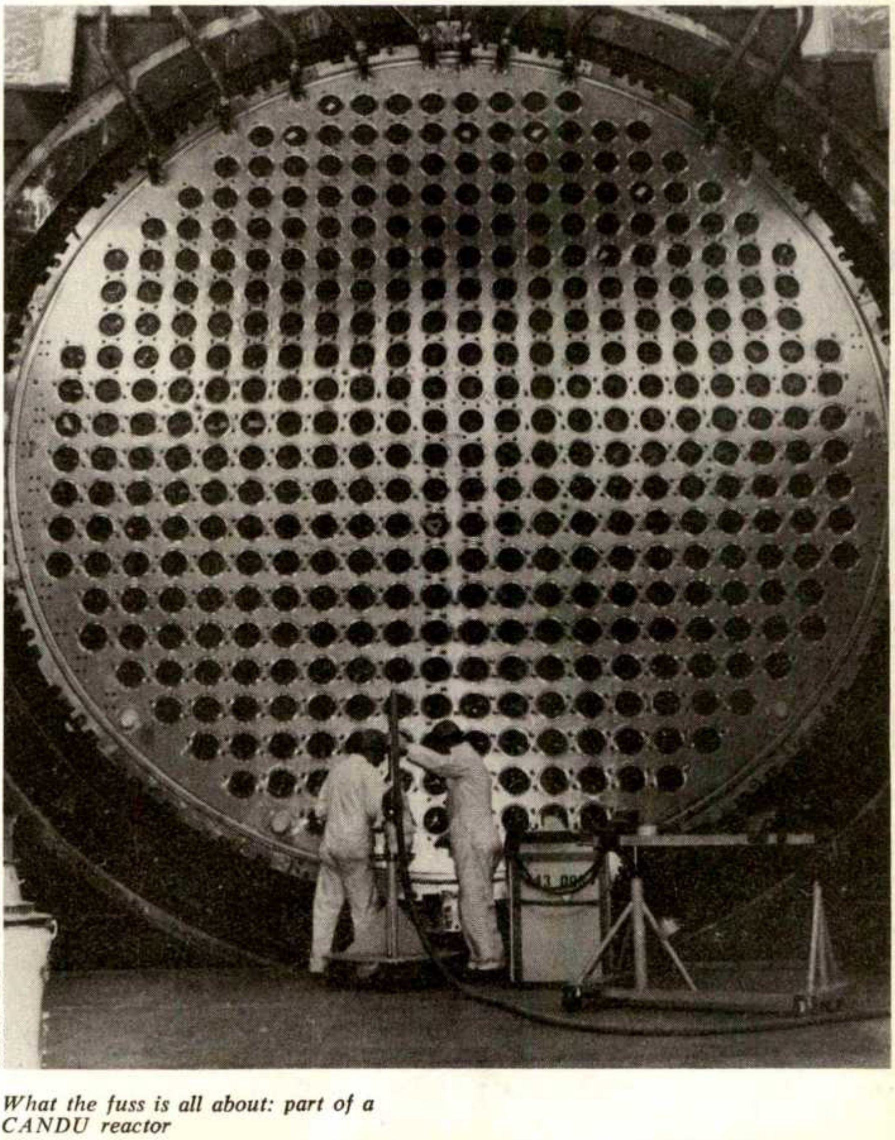


Photo: IPC

What the fuss is all about: part of a CANDU reactor supply further nuclear assistance without assurance that plutonium coming from it will not be used for further nuclear explosions. It is the lack of that assurance which is chiefly responsible for last week's announcement. The Canadian government has wanted to upgrade the safeguard system which already exists on RAPP-II, which India is capable of finishing alone, though it will take longer without Canadian help.

According to Mr MacEachen, the question the government has been faced with is whether it is better in the interests of non-proliferation to get out of nuclear co-operation with India completely, risking a collapse of current safeguards, or to complete the project while upgrading the RAPP-II safeguards. The crucial point, however, concerns the absence of safeguards on the very reactor that produced India's first nuclear explosion—the CIRUS. CIRUS would not have been covered by the agreement, Canadian officials apparently thinking it unimportant because it produces only enough plutonium to make one or two bombs a year. Furthermore, future reactors now being built or planned by India to Canadian design would not have been safeguarded by the terms of the Canadian agreement.

Before the latest decision the rationale that the government was using seemed to ignore the very rationale Frank Maine had put forward to defend it: that, because the RAPP reactors are designed to produce power, they could only be used to produce weapons-grade plutonium at the expense of power production. On the other hand, a research reactor like CIRUS is ideal for producing fissile material. That, in fact, is what was done two years ago.

Another question critics asked was how much Canada could rely on safeguards even if they were agreed to. Canada thought it had agreement from India that she would not explode a nuclear device before she actually did so. "Safeguards," said T. C. Douglas, "are worth nothing unless the people who give them are the kind of people on whom we can rely." Robert Stanfield, the former opposition leader, wondered if "the government and those who are advising the government are not acting on the premise that these potential customers are going to get the bomb anyway, and Canada might as well get the business".

There has been some indication that a firm stand by Canada does have some effect. South Korea cancelled a fuel reprocessing plant under pressure from the United States and Canada when Canada threatened to cancel construction of its nuclear plant. Pakistan has contracted with France to buy a processing plant—for reasons unsatisfactory to Canada—and Canada was hoping that if its agreement with India was successfully concluded it would obtain a similar agreement with Pakistan. Such an agreement would prevent Pakistan from using explosive devices from waste fuel from a Canadian-designed plant in Karachi. Until it receives a commitment, Canada will not supply Pakistan with a plant to make fuel bundles for the Karachi reactor, and has threatened to hold back delivery of fuel made in Canada.

What the Canadian nuclear export critics have been saying is that Canada should undertake a moratorium on sales of nuclear materials for a period, during which pressure should be exerted on other exporting countries to agree on adequate safeguards, with sanctions. With respect to India at least, the Cabinet has now made a decision, and through its external affairs minister the Indian government has already expressed its regret and disappointment. The full background behind the Canadian announcement has yet to emerge—India quickly disclaimed any responsibility on her part—and the forthcoming meeting of the "Group of Seven" has now taken on added interest. ☐

CHEMICAL WEAPONS

Slow progress to disarmament

Atttempts are being made to reduce, through international understandings, the chances of chemical warfare. John Stares outlines some of the problems

Drawing up an international treaty banning chemical weapons ought to be a simple matter. Chemical warfare has been illegal for more than 50 years. No chemical weapons have been manufactured, at least in the West, for almost 10 years, and some countries have begun to dispose of their obsolete stocks. Admittedly a new generation of chemical weapons—the binary weapons—is being developed in the United States, but the US Congress has so far refused to grant funds for their manufacture. And yet, in almost 10 years of international negotiations, the Conference of the Committee on Disarmament (CCD) in Geneva has failed to produce a chemical disarmament treaty.

As time goes on, the need for a chemical warfare (CW) treaty becomes more urgent. Details of the chemistry of CW agent production are widely known, and there is a growing risk of the proliferation of chemical weapons. Advances in our knowledge of biochemistry, and developments in genetic engineering, bring with them the danger of the development of new CW agents. It may only be a matter of time before the US Congress does grant procurement funds for binaries. If proliferation, the development of new agents or procurement of binaries does become reality, the problems of reaching agreement on chemical disarmament will increase considerably. But in a major speech to the CCD last month, the US delegate, Joseph Martin, indicated that agreement is still a long way off.

Ambassador Martin said that there are several issues where, in the US view, "an adequate basis for forming judgments already seems to exist, and where agreement may be possible in the relatively near future". On the question of the scope of a CW treaty, he said that "the complete prohibition of chemical weapons cannot be realised in a single comprehensive agreement", and that therefore there should be a phased approach to chemical disarmament. A step-by-step approach is not a new idea, but while in the past the aim was to concentrate initially on the most dangerous "supertoxic" CW agents, such as the nerve gases, it is now generally agreed that an initial CW treaty should encompass all lethal CW agents.

Thus the phased approach to chemical disarmament should be based on the activities to be included in the ban. Initially attention should be focused on banning the production of all lethal CW agents, and on reducing existing stockpiles, although exactly what measures should be applied to stocks—whether, for example, the treaty should require destruction of all, or only part, of the stocks—is still a matter for debate.

On the question of the definition of CW agents, it is the "tentative view" of the USA that it might be adequate to rely on a general purpose criterion—perhaps along the lines of the formulation in the 1974 Japanese draft CW treaty, where CW agents were defined as chemicals "of types and in quantities that have no justification for protective or other peaceful purposes"—and on lethal toxicity standards. Other criteria, such as representative lists of agents, or structural formulae, could perhaps be used as well, if necessary.

To have taken five years to reach a consensus that, in an initial CW treaty, production of all lethal CW agents should be banned, that lethal CW agents can be defined satisfactorily, and that some, as yet still unspecified, measures should be applied to existing stockpiles, is not exactly impressive progress—particularly when there is still no agreement on how to ensure compliance with such a treaty. Verification remains the major stumbling block to the conclusion of any CW treaty, partial or otherwise.

In recent years, a mass of detailed information on technical methods of verification has been produced by SIPRI, Pugwash and other groups, and presented in the CCD. It has been suggested that therefore all the elements necessary to build an effective CW treaty are now available, and that all that is needed is a political decision to conclude the treaty. But Ambassador Martin stressed that the USA does not share this view, maintaining that effective solutions to the problems of verification have not yet been found.

A first stage CW treaty would require a verification system capable of providing adequate assurance that CW agents are not being produced, and that the destruction of stockpiles actually complies, qualitatively and quantitatively, with the provisions of the treaty. There are two broad aspects of verification. First, the methodology. The methods studied include, for example, statistical monitoring of data on the production, transport and use of phosphorus and phosphorus compounds, a technique aimed at detecting possible diversion of phosphorus from civilian industry to military uses; and sophisticated scientific analytical techniques capable of detecting organophosphorus compounds in air, water or soil samples (and thus of detecting production of nerve agents), and of monitoring destruction activities, from remote locations. In addition, reconnaissance satellites might be used to observe mothballed production facilities and hence warn of any resumption of activity.

These methods are certainly not foolproof. But, as Martin admitted, 100% effective verification is not necessarily essential: : what is needed is a system capable of setting the difficulties of evasion high enough to deter treaty violation. These methods, especially when used in combination, could form a useful basis for such a system. And the methods are constantly being developed and refined: SIPRI, unfortunately, seems to have lost interest in chemical disarmament problems, but other groups, such as the Pugwash Chemical Warfare Study Group, are actively engaged in studies on this question.

The other aspect of verification is the implementation of the system. This is a purely political decision, involving an assessment of the adequacy of the degree of assurance that could be provided by the technical verification methods. Martin made the US view quite clear, that in order to obtain adequate assurance of compliance with the provisions of a CW treaty, there must be provision for supplementing the technical verification methods with on-site inspection of production and destruction facilities, whether on a mandatory or a challenge basis.

On-site inspection has always been a politically sensitive issue, and it is unlikely to be resolved until there is a considerable improvement in international trust and confidence. Of course, in many ways, this is a circular argument: the greater the degree of international confidence, the easier it would be to implement a verification system including on-site inspection, while at the same time the need for extensive verification would decrease. Nevertheless, there is a point at which the degree of international mutual confidence is such that a given level of verification, including on-site inspection, is both perceived to be necessary, and is acceptable, politically, by all sides.

The international chemical disarmament negotiations therefore seem to have reached the stage where, all the technical elements of a verification system being available, the emphasis is on building international confidence. In this context, Ambassador Martin made the valuable suggestion that it "might be useful to consider the feasibility and utility of technical exchange visits to selected chemical production and disposal facilities in different countries". This was an idea that had been emplanted last year within the US administration, and those of other countries, by the Pugwash Chemical Warfare Study Group. It is encouraging that its public endorsement by Ambassador Martin coincided with the Group's latest Workshop, held in London and attended by experts from 11 countries, East, West and non-aligned.

There have been indications, unofficially, that other countries, including the Soviet Union, would respond favourably to this idea. If a programme of visits could be arranged, at which it would be possible to test the methodology of verification in practice, this would represent a significant step forward in the chemical disarmament negotiations. ☐

ASBESTOS

Tiny straws in the wind

Recent events in Britain have raised anew questions about the true extent of the asbestos health threat. Allan Piper reports on signs that white asbestos may, after all, be just as dangerous as the blue variety, and that the hazards stretch beyond the risk of asbestosis on the factory floor

THAT asbestos is a threat to health is nothing new—the progressive respiratory disease known as asbestosis led to the first asbestos regulations for industry fully 45 years ago. But the latest controversy in Britain has done more than raise questions about the adequacy of the regulations now covering its industrial use; it has also served to publicise the possibility that asbestos poses a threat to the public at large.

For asbestos does not just cause asbestosis. It is also a carcinogen. Minute asbestos fibres, if inspired, can pierce individual cells to cause both cancer of the lung and mesothelioma, a particularly malignant, inoperable cancer which attacks the lining of the pleural cavity. There is also emerging evidence that fibres can migrate across internal membranes: they have been detected in cancerous liver tissue, and may also be able to infiltrate the gastrointestinal tract.

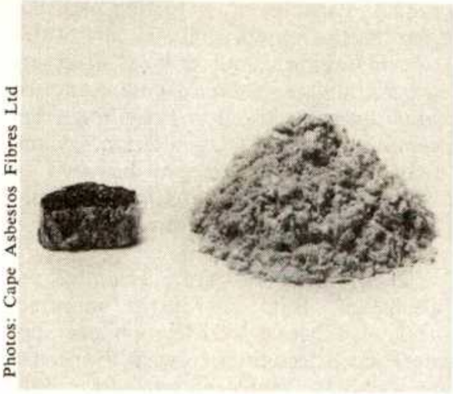
It was only at the end of last month that some of the crucial issues became fully public, when an eight-year-old Department of Health (DHSS) report (together with a revised version) was suddenly made available in the Parliamentary Library following a question in the House of Commons. The report, which was prepared for the DHSS Standing Medical Advisory Committee by a working party it established under the Chairmanship of Sir Richard Doll (then Director of the Medical Research Council's Statistical Unit in London, and now Regius Professor of Medicine at Oxford), clearly states that minimal levels of exposure to asbestos pose a real cancer hazard. According to the report the dangers are not necessarily limited to the naturally fine, blue asbestos, crocidolite, as has hitherto been maintained; they probably extend also to the extensively used white asbestos, chrysotile, which is now milled far more finely than in the past—thus posing a far more widespread threat than previously believed (a possibility which the report clearly acknowledges).

In that light, the official view that the dangers of asbestos have remained confined to industry seems complacent. Even regarding the situation within industry, the suggestion that the cancer threat was removed with the exclusion of crocidolite under the updated Asbestos Regulations of 1969 seems worrying now that the DHSS report has identified chrysotile as a possible hazard. Moreover, the report also called for a review of the issues once the 1969 regulations had been in force for five years. That dateline came up last May, but the review is only now getting under way—and not because of the report itself.

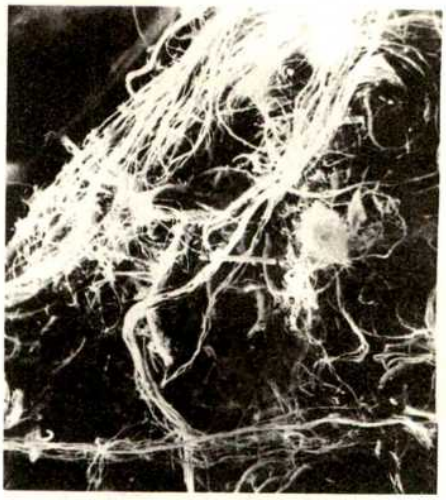
How far the authorities should have moved towards enforcing more stringent regulations remains somewhat arguable, however, if only because the broader scientific community can provide few helpful clues which might finally determine the true extent of the dangers of asbestos. A major difficulty arises because there have been few sufficiently long-term studies. Asbestos fibres can only be detected in diseased tissue using electron microscopy, and the complication means that until now they have been sought only in cases known to be associated with asbestos. Consequently, epidemiological studies—investigations of disease patterns throughout the population—are lacking. In fact, the situation remains so unclear that different interpretations of such data as exist are leading to a rift within the involved scientific community.

Extremes of opinion about the report's claims are easily found. One Medical Research Council (MRC) scientist told *Nature* there was "no evidence at all" of any cancer risk to the general population as suggested in the DHSS report. He was also sceptical of the suggestion that chrysotile may be as dangerous as crocidolite. These rejections came even though a colleague at the same unit has published data showing that of 120 mesothelioma cases in a single study, 23—the largest single statistical unit—were attributable to chance exposure to minimal levels of chrysotile.

On the other hand, a research worker in London fully backed the DHSS report, and even went on to suggest that glass fibres and commercially available talcum powder should also be studied as possible carcinogens. These materials could pose a cancer threat similar to that presented by asbestos since it is the size of the fibres, rather than any chemical agent, which is suspected of inducing cancer. In fact, a recommendation on minimum fibreglass diameters has already gone out to industry from one MRC unit. *Nature* was also told that asbestos fibres in the form of anthophyllite have already been detected in studied samples of talcum powder, but a member of the MRC's Pneumoconiosis Unit at Penarth refutes this, claiming that extensive studies on talcs from all over Europe have failed to reveal a single fibre.



Photos: Cape Asbestos Fibres Ltd



White asbestos or chrysotile (above), and (right) Stereoscan photograph of Chrysotile fibre (magnification ×4500)

The hope is that the ongoing research effort will help resolve the controversy. In Britain work at six MRC units ranges from a major co-operative programme with the Ministry of Defence (into asbestos hazards in naval dockyards), to studies of the aerodynamic properties of fibres. Further MRC laboratory studies of glass fibres are also planned to accompany research already in progress under the sponsorship of the DHSS Health and Safety Executive (HSE), while a major, year-long epidemiological study of all mesothelioma cases should soon be under way at Penarth. In addition, several projects are supported in British universities. On the international front the World Health Organisation's Agency for Research on Cancer announced plans last January for a coordinated epidemiological study on fibre glass workers in Europe; and when its Advisory Committee on Asbestos Cancers meets later this year more research recommendations can be expected.

But while the research efforts continue, the outstanding need for new legislation, and suitable enforcement, remains. The DHSS report underlines the inadequacies of the 1969 regulations, which do not cover the public beyond factory workers and do not recognise the newly defined cancer hazard. Yet no new legislation is planned beyond a marking scheme to cover do-it-yourself asbestos materials. The current review may recommend more, and even the adoption of the recommendations of the DHSS report would represent a considerable move towards eliminating the general dangers. It would mean the eventual disappearance of crocidolite (which still enters Britain inside manufactured goods), the early introduction of suitable asbestos substitutes, tight controls over the sale, use and disposal of asbestos, and possible checks on general emission levels by the Alkali Inspector.

One difficulty is that the official statistics do not reinforce the case for such action. DHSS figures show only 800 recorded cases of asbestosis, while recorded cases of asbestos cancer and mesothelioma are negligible. But whether the numbers mean very much is questionable on several counts. In the first place, past ignorance of the general danger has ensured that many asbestos cases are attributed to something else (or interpreted as secondary cancers). Industrial settlements out of court provide another distortion. And then there is the hidden factor arising because mesothelioma can take up to 50 years to develop, whereas the asbestos dangers have become significant only during the past 30 or so.

The industry disputes these arguments, and points to a national factory survey by the Health and Safety Executive under existing regulations. Critics regard that move scornfully, particularly after the recent Parliamentary Commissioner's report questioning the vigilance of the Factory Inspectorate at Acre Mill in Yorkshire, the site of an asbestosis tragedy. But they are confronted by a powerful and well-organised industrial lobby, and equivocal union attitudes.

Meanwhile, many unacknowledged asbestos victims (or their families) go uncompensated. Questions about the dangers of asbestos, and about how to minimise them, have been asked and almost forgotten before. At least now it looks as though the latest resurgence of concern could be sustained until answers are found. ☐

IN BRIEF

Nuclear trade . . . continued

The trends in international nuclear trade were further emphasised by developments last week. South Africa apparently hopes to place the $1,000 million dollar contract for its proposed twin reactor nuclear power station by the end of the month. A consortium including General Electric of the USA, Brown Boveri of Switzerland and Rijn-Schelde-Verolm of Holland is the leading candidate if credit guarantees can be arranged—a matter now troubling the Dutch coalition government.

Following agreement in principle three months ago in Nice, France and West Germany have now agreed on the practical details under which the French Atomic Energy Commission and the German companies Interatom and Kraftwerk Union will exchange ideas and expertise for a standard marketable design for a fast breeder reactor. Little progress has so far been made on cooperation in the high temperature reactor field, however. Japan–USSR cooperation in the nuclear field, first mooted in discussions last year, has taken a step forward with the news that Japanese nuclear plant manufacturers are to submit estimates for a proposed Soviet project.

Among other developments: Westinghouse has reportedly signed a letter of intent to sell Egypt one of the two nuclear reactors contained in last year's US–Egypt deal; Kuwait is to seek tenders for a 40–60 MW training reactor as part of its programme for twin 1300 MW stations eventually to provide electricity and desalinated water; and Korea has confirmed that it has not so far approached Britain on nuclear cooperation. Meanwhile, 13 members of the 19-nation International Energy Agency have agreed to exchange information to help insure nuclear reactor safety.

Britain's uranium difficulty

The worldwide scramble for scarce uranium supplies has landed the UK Government in the centre of an embarrassing controversy over a 1970 contract to import an annual 1,500 tonnes of uranium from Rio Tinto Zinc's Rossing mine in the politically-sensitive territory of Namibia. Under strong UN pressure to cancel the deal, the Labour Gogernment is also burdened by a promise to withdraw which it made while in opposition during 1973. Namibian uranium is arguably crucial to Britain's nuclear future: projected 1980 requirements for the UK's first and second generation reactors stand at over 2,000 tonnes a year, and on this point the debate concerns whether, in the face of fierce international competition, UK demands can be met as cheaply or reliably by alternative producers.

Canada's energy moves

In a drive towards energy sufficiency by 1985 Canada is to introduce incentives to boost exploitation of gas and oil reserves, change its licensing system and give the state-owned company Petro-Canada preferential powers over other companies.

Organised research

KENNETH MELLANBY

ALEXANDER Solzhenitsyn is generally admired as a writer, and as the winner of a Nobel Prize for Literature. His political views are more controversial. In Western countries some of those who have watched his recent interviews on television say they want action at once, to combat the dangers of Soviet imperialism and communist infiltration; others think his views exaggerated and irrelevant to democratic countries. His novel *The First Circle* deals with the political situation in Russia under Stalin in 1950, but it also contains something which may be relevant to the debate about the organisation of scientific research, a debate which is continuing today in Britain, in many European countries and in North America.

The book describes the Mavrino Special Prison, which is in effect a government research laboratory. Many of the inmates are distinguished scientists and technicians who are alleged to have collaborated with the Germans when prisoners of war. In addition there are "free" workers, mostly in junior positions. The establishment is under the direction of scientists who have not yet incurred the displeasure of the regime, with close supervision from government ministers with more or less scientific experience. The purpose of the research is mostly highly practical, generally aimed at improving the surveillance of those suspected of ideological deviation, though sometimes the research workers may become involved in scientific problems and forget how their results may be applied. It is generally understood that any prisoner whose work is successful will have his sentence remitted; this is intended to ensure his "motivation".

Much of *The First Circle* concerns politics and the problems of insecurity and intrigue in Stalinist Russia, and is thus not, we hope, directly relevant to laboratories in other countries. Yet the organisation of the research has, in some instances, a disquieting resemblance to the system now being developed in Britain and elsewhere—though in most cases the resemblance is that of an obscene caricature. However, such caricatures have sometimes given a foretaste of future developments.

Thus at Mavrino we see bureaucracy encroaching everywhere, and an obsession with paperwork. Instead of getting down to actual research, the organising scientists are concerned with "plans, plans, promises and more plans". The scientists as a whole have little influence on the choice of the subject to be investigated, and are often compelled to devote their efforts to topics they know to be worthless. The research programmes are controlled by rigidly described "projects" with an exact—and often impossible—timetable for their completion. When, as is often inevitable, the required results are not forthcoming, a crash programme (described by one of the participants as "heads down and charge") is mounted. As a result we read of "the rushed, sloppy, careless style of work which was the rule" at Mavrino. The whole system is seen to be remarkably inefficient, unable to produce even short term results.

Notwithstanding (or even because of) Solzhenitsyn's gloomy predictions, we may avoid the political fate he warns us against. But I think we can also learn from his account of Stalinist scientific research. Changes in the organisation of science in Western countries seem to be introducing many of the methods used at Mavrino. Can we be sure, even under a democracy, that they will not reduce even further our scientific originality and productivity?

correspondence

Jukes on food

Sir,—Thomas H. Jukes, writing on nutrition (May 13, page 92), tried to be funny at the expense of accuracy. To give but one example, his response to the recommendation from the Royal College of Physicians' Working Party that less butter should be eaten, is that it is "just as logical" to place a warning on "every nubile woman, for human milk contains fats similar to those in cow's milk and we are told the pre-arteriosclerotic changes can start at a very young age".

Really, Mr Nature, this sort of stuff will not do. Cow's milk is suited to body growth, with three times as much protein and mineral as human milk which, by contrast, is suited to brain, vascular and organ biochemistry. Human milk contains between 4–7 times the amount of essential polyunsaturated fatty acids of 18 carbon chain length, and some 8–12 times the amount of long-chain polyunsaturated fatty acids (C20–C22), which are the principal essential fatty acids found in the brain, artery, endocrine and reproductive systems of the body. Perhaps Thomas Jukes had not noticed that the brains and arteries of the human are somewhat differently developed as compared with those of the cow.

M. A. Crawford
The Zoological Society of London

Government and industry

Sir,—Since you have dealt editorially (April 29) with the difficult question of salary comparisons between industry and the civil service, I would seek space to make only two points in reply to Harold Turner (April 8, page 472).

First, I agree with him that the problems of British industry are not primarily technological, but economic and managerial. My article (February 12, page 436) was intended to be a constructive contribution to solving these more serious problems. Second, the CBI has devoted considerable thought to defining the optimum relationship between government and industry in the field of research and technology, and last October sent a fairly lengthy, and generally well-received, document on the subject to appropriate parts of the government machine.

There is no simple formula, but essentially the CBI sees the need to develop government–industry technological relations through such mechanisms as the Requirements Boards of the Department of Industry, and to establish links between these Boards and the Economic Development Committees which operate under NEDC in order to relate technological to economic factors.

D. W. Budworth
Confederation of British Industry

Isolative sound-change

Sir,—Your correspondents Alan Ross (April 22, page 664) and Ian Hurrell (May 13, page 93) discuss the problem of vowel-shifts with particular reference to the evolution of the English language. We must first of all be certain that a problem exists.

"Anglo-Saxon" is the name given to the miscellany of people who migrated to the British Isles from all of the coastal regions around the North Sea—from what is now Flanders to northern Scandinavia. The languages and dialects of these peoples, which fused to become the dialects of the Anglo-Saxon language, were related but diverse. Both Anglo-Saxon and Middle English were highly heterogeneous languages, even when only the literary forms are considered (compare the language of *Beowulf* with that of the Exeter Book in the Anglo-Saxon, or compare the original language of *The Canterbury Tales* with that of *Piers Ploughman* or of *Sir Gawain and the Green Knight*, all three of which were written in the late fourteenth Century).

As England became united and contact between dialect groups increased, some words from each dialect would survive but most would vanish, a process that has continued to this day. The literary language at any one time was a reflection of the region of medieval England which was dominant at that time. Either of these two factors would give the impression, judging only from the literary forms which have survived, that well defined shifts in pronunciation had taken place, whereas the changes that did occur were due to chance.

In order to show that a problem in "psycho-linguistics" exists and that the explanation is not trivial, one must show that:

- all dialects of the language, not merely the literary form, changed in the same direction at the same time
- all words in any one dialect with a particular vowel sound changed to another sound (for example, ā→ǭ)
- these changes were not related to political changes, especially with respect to the region of origin of the royal household.

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Tropical trees

Sir,—May suggested (*Nature*, **257**, 737–8; 1975) that "relatively stable tropical environment(s)" tend to produce plant species of high competitive ability (K-selected) as opposed to opportunists (r-selected species) which he considers are favoured by "relatively vagarious, temperate climates". This generalisation is misleading. It overlooks the class of trees, also well-represented in tropical rain forest, known as pioneers which *are* opportunists. These are the species which colonise gaps. They possess a whole syndrome of features which equip them to this niche in the ecosystem.

In brief, they possess small, easily (wind or animal) dispersed seeds which have dormancy and are produced more or less continuously; are extremely fast growing, unable to regenerate under shade, and have pale wood of low density. Balsa (*Ochroma lagopus*) of Latin America is the classic example. Appreciation of the existence of two classes with strongly contrasting biology is vital to an understanding of both the ecology and silviculture of tropical rain forest. For example, it has been realised for some time that the most valuable African rain forests in which timber species of Meliaceae (African mahoganies) dominate are a late seral stage, not replacing itself but altering to a forest with less valuable and smaller trees of different families; and the *Campnosperma* and *Terminalia* forests which are the main natural resource of the Solomon Islands are stands of near-pioneers.

Pioneer species are the ones foresters nowadays mainly plant. Their biology and timber properties suit modern conditions. The humid tropics are well-suited to rapid timber production and in fact tropical pioneer trees will be one of the world's main timber sources by the turn of the century. It is therefore important to correct any misconception that there are no pioneers in tropical rain forest.

T. C. Whitmore
University of Oxford, UK

news and views

Viral infection mimicking genetic patterns

from Arie J. Zuckerman

THE recognition of hepatitis B surface antigen (originally known as Australia antigen or Au antigen) and its homologous antibody as two specific markers of infection with hepatitis B virus resulted in considerable progress in the understanding of the epidemiological, clinical and immunological behaviour of this infection. A major public health problem that has emerged from the results of numerous surveys is the remarkably high prevalence of persistent carriers of the hepatitis B surface antigen in many areas of the world, resulting in an estimate of over 110 million carriers. For practical purposes, a persistent carrier had been defined as an individual in whom the surface antigen has been detected repeatedly for more than three months. The duration of carriage may be many years (Zuckerman and Taylor, *Nature*, **223**, 81; 1969) but some carriers may lose the antigen after a shorter period of time (Szmuness *et al.*, *Am. J. Epidemiol.*, **98**, 104; 1973).

The reported percentage of carriers varies widely in different geographical areas, from less than 0.1% to as many as 20% of the apparently healthy population. The carrier rate is higher in warm-climate countries than in the temperate zones, higher among males than females, higher among children and adolescents than among adults, and higher among urban than among rural communities. The underlying mechanisms for the establishment of the persistent carrier state have remained largely unknown, although the subject of many investigations. Blumberg and his colleagues (*Proc. natn. Acad. Sci. U.S.A.*, **62**, 1108; 1969) proposed that susceptibility to persistent asymptomatic carriage of hepatitis B surface antigen was based on a simple autosomal recessive gene (Au^1), once infection with hepatitis B virus had occurred. This gene was considered to be common in tropical areas but rare in temperate zones. Individuals homozygous for this gene (Au^1/Au^1) would have persistent antigen without overt manifestation of hepatitis, although they remain as carriers of the associated virus, whereas heterozygotes (Au^1/Au^0) and individuals without the gene (Au^0/Au^0) would not remain as carriers. This hypothesis has evoked considerable controversy (*Nature*, **248**, 377; 1974). Data now obtained by Stevens and Beasley (*Nature*, **260**, 715; 1976) and Mazzur (this issue, page 316) are not compatible with this simple genetic hypothesis. Stevens and Beasley determined the rates of hepatitis B surface antigenaemia among children in Taiwan, which is a high prevalence area, in families with two antigen carrier parents and compared the rate with those with a carrier mother and an antigen-negative, antibody-positive father. The results suggest that all the children could become antigen carriers, regardless of the genetic background. Furthermore, acquisition of the carrier state was probably influenced by the dose of virus received by the babies (Stevens *et al.*, *New Engl. J. Med.*, **292**, 771; 1975) and the age of infection (Szmuness and Prince, *Am. J. Epidemiol.*, **94**, 585; 1971). From Mazzur's data on the children of matings between two carriers or between one carrier and one non-carrier the genetic hypothesis can also be rejected. In this study there were, for example, three families in which both parents were antigen carriers, yet only 2 of the 13 children in this group were carriers. Three of the children had antibody and therefore they had been infected with hepatitis B virus but they did not become carriers, which is clearly incompatible with the genetic theory. The remaining eight children had no serological evidence of infection. All four children in another family were carriers, although the antigen was not detected in either of the parents. The probability of this occurrence according to the simple genetic hypothesis was calculated as 1 in 256.

More complex genetic explanations have also been proposed, including a link between heterozygosity for Gm antigens and increased susceptibility to persistent antigen carriage. Homozygosity for Gm antigens on the other hand leads to an increased probability of developing surface antibody when infected with hepatitis B virus (Blumberg *et al.*, *Nature*, **236**, 28; 1972). This aspect was not supported by the investigation of Schanfield *et al.* (*Nature new Biol.*, **243**, 81; 1973) in normal blood donors. In a subsequent multicentre study, Schanfield and associates (*Int. Symp. Viral Hepatitis. Devl biol. Standard*, **30**, 257–269, S. Karger, Basel, 1975) found no association between the Gm phenotype and susceptibility to hepatitis B virus, using the surface antigen or the ability to make antibody to it as a marker. It was concluded that variation in the distribution of Gm haplotypes did not seem to be a feasible explanation for variation in surface antigen and surface antibody frequencies in a series of multiply transfused populations in the United Kingdom, Cyprus, Greece and Sardinia.

Another possible genetic association has been described more recently, however. Boettcher and colleagues (*J. Immunogenet.*, **2**, 151; 1975) established an association between an HLA antigen and the presence of hepatitis B surface antigen in a group of Australian aborigines. There was a statistically significant deficiency of W 15 among carriers of the antigen and this was explained in terms of linkage disequilibrium between W 15 and an immune response gene involved in clearing the surface antigen from the body. Although it was pointed out that the hypothesis that becoming a carrier is largely genetically determined is an oversimplification, it was accepted that the potential for becoming a carrier is a recessive condition controlled by a single autosomal gene. It was proposed that if the antigen W 15 is a (hepatitis B) virus receptor on cells, and it is disadvantageous to have viruses located on these receptors on certain cells, it would be of selective advantage also to possess the immune response gene responsible for clearing viruses from the receptors. In these circumstances, association of W 15 and the hepatitis B surface antigen immune response gene would be of selective advantage. Indeed, the major complex of histocompatibility antigens has been shown to be closely associated in animals and in man with the immune response genes. This could explain the association of immunological diseases with HLA antigens, for example on the basis of a possible receptor role of HLA antigens or the "molecular mimicry" between viruses and HLA antigens. Vladutiu (*Lancet*, **ii**, 288; 1974) pointed out, however, that lymphocyte-defined determinants have been shown to exhibit a stronger association with diseases than serologically-defined determinants. He also noted that the enthusiasm generated by the idea of association of HLA antigens with various diseases is not unanimous, and although there is a strong association with a few diseases, the underlying mechanism remains unknown. Vladutiu stressed that there are many pitfalls when searching for such associations and that the principal source of errors is the statistical analysis. Another example cited is the association of many diseases with HLA 1 and HLA 8. Although these antigens are absent in Japanese, diseases known to be associated with them are seen among the Japanese population. It is apparent, therefore, that further studies and experimental models are necessary.

Finally, it is widely recognised that family clustering may be entirely a function of an increased risk of exposure to an infecting virus rather than genetically determined susceptibility; particularly if a parent is excreting the virus and more so if the mother is the transmitter. Mazzur notes that persistent infection with hepatitis B virus may be representative of other infections which may also mimic genetic patterns. Indeed she suggests that perhaps polygenic birth defects and other diseases, which the currently accepted as genetically determined because of family clustering may also be caused by perhaps as yet unrecognised infectious agents. This suggestion merits considerable further investigation and careful evaluation of the results. □

Theileria in the laboratory

from F. E. G. Cox

THE increasing problem of protein shortage in Africa has once again concentrated attention on the parasitic diseases of cattle and although recent attempts at vaccinating animals against these diseases give grounds for optimism (*Nature*, **260**, 380; 1976) much basic research still remains to be done. East Coast Fever, caused by a protozoan *Theileria parva* and transmitted by a tick *Rhipicephalus appendiculatus*, still kills up to 40% of calves over a wide area of Central and East Africa. The parasite develops through two cycles of multiplication in lymphocytes before entering red blood cells and becoming infective to the tick. Only cattle, buffalo and Indian water buffalo can support the infection through to its final infective stages and research has been limited by the non-availability of a suitable laboratory host. In 1970 some progress in the maintenance of *T. parva in vitro* was made (Malmquist, Nyindo and Brown, *Trop. Anim. Hlth Prod.*, **2**, 139; 1970) when it was shown that the early stages of the parasite in lymphocytes could be grown in a bovine cell line but the infection did not proceed through to the blood forms. More recently the use of interspecific heterokaryons formed by the virus-induced fusion of cells has found favour (Irvin *et al.*, *Int. J. Parasitol.*, **4**, 519; 1974) but still no invasion of blood cells has been recorded.

In this issue of *Nature* (page 311) Danskin and Wilde describe how they have been able to grow all stages of *T. parva* up to the blood forms in culture. Their technique is simple; they use the modified Eagle's medium described by Malmquist *et al.* with 20–30% foetal calf serum (instead of 10%) and in addition 5–10% bovine lymph. The usual macroschizonts and microschizonts developed and bovine blood cells added after 18–66 h became infected but not those added after 90 h. The number of cells infected, 0.1%, was low but cattle are infective to ticks at this level of parasitaemia.

The next stage will be to infect ticks from cultures and considerable progress has been made on an understanding of the feeding biology of *R. appendiculatus* by Tukahirwa (*Parasitology*, **72**, 65; 1976). The larvae and nymphs require 8–9 d after hatching or moulting before they can feed and this they do for 4–6 d. The adults cannot feed for 6–9 d after moulting but eventually feed for 7–9 d. On *Theileria lawrenci* (a parasite closely related to *T. parva*, so realistic comparisons are possible) adult *R. appendiculatus* infected as nymphs become infective to cattle 3 d after beginning to feed and remain so for the next 6 d (Young *et al.*, *Parasitology*, **71**, 27; 1975). These authors were able to correlate infectivity with morphological criteria of parasite maturity in the ticks' salivary glands so it should be possible to infect nymphs from cultures, allow the nymphs to moult, let them feed on rabbits and collect infective stages to initiate further cultures after 3–4 d using the morphology of the parasites as a guide to infectivity. Experimental cattle should no longer be needed and basic research will not necessarily have to be done in Africa. This should please the growing number of scientists who are concerned about excessive animal experimentation.

Diseases caused by *Theileria* are not confined to Africa and in India considerable progress is being made in the understanding of the biology of *T. annulata*. Bhattacharyulu, Chaudri and Gill (*Parasitology*, **71**, 1; 1975) have confirmed that *Hyalomma anatolicum* and other ticks transmit this disease, that the infection passes from larvae to nymphs and nymphs to adults and that the infection is transmitted during the first 24 h after feeding. Of considerable interest is the finding that if infected larvae are fed on cattle as nymphs the adults are not infective whereas if the nymphs are fed on rabbits which are not susceptible to infection with *T. annulata* the adults are infective. It seems as if the infective stages are "used up" during feeding on an appropriate host. This may call for some reappraisal of the experimental procedure of using "susceptible" and "non-susceptible" hosts almost randomly in various fields of research into piroplasmosis. The use of cultured parasites would avoid this problem. □

Of gods and men

from David W. Hughes

STARS were first named by the herdsmen, hunters, sailors and travellers who lived along the banks of the Euphrates and Ganges, and it was these people and not the learned and scientific who were the sideronomists. The star names used today have been gradually accumulated over the last three thousand years or so. They have come in the main from Greece, Rome and the nomadic and scholarly tribes of Arabia. Castor and Pollux (α and β Geminorum)—the heavenly twins; Regulus (α Leonis)—the ruler of the affairs of the heavens; Betelgeuze (β Orionis) from *ibt al jauzah*, the armpit of the central one, Algol (β Persei) from the Arabian's *Ra's al Ghul*, the Demons head, are a few examples.

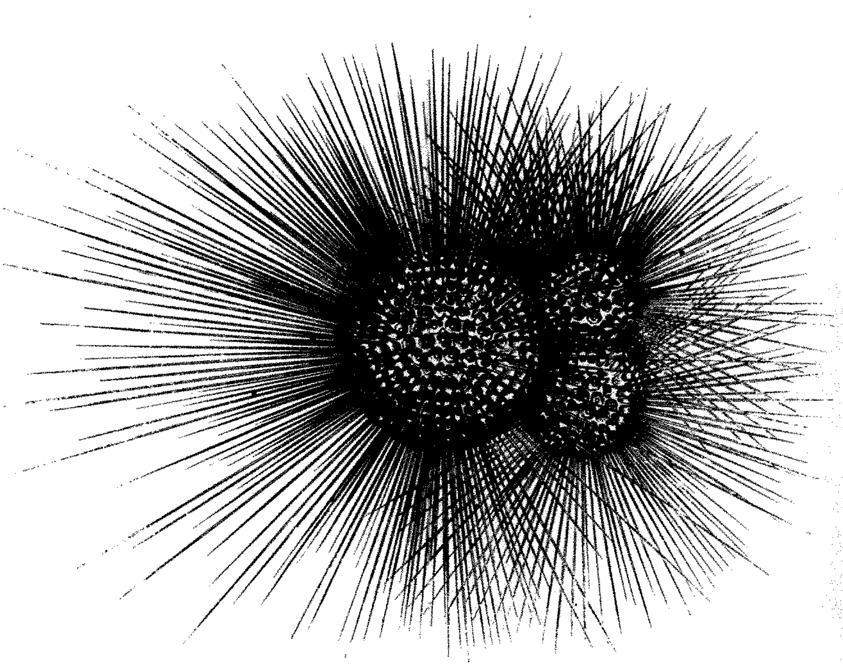
Today naming stars has gone out of fashion and astronomers make do with rather dry titles such as V1057 and L789-6. However naming say, Jovian satellites and the features of planetary surfaces that have recently been revealed by imaging devices on space craft is very much in the news and a subject of some controversy.

Carl Sagan (Laboratory for Planetary Studies, Cornell University) discusses this problem in his recent paper in *Icarus* (**27**, 575; 1976). In 1967, confronted with enormous amounts of new data relating to the near and far side of the Moon, Commission 17 of the International Astronomical Union set up a subcommittee on lunar nomenclature. They decided to extend the previous system of nomenclature to the reverse side of the Moon and to name craters after deceased scientists, special attention (and presumably preference) being given to astronomers. It was also proposed (*Trans. IAU*, **XIIIB,** 103; 1967) that the nomenclature should be widened to include people associated with the present space effort and particularly cosmonauts and astronauts and others who have lost their lives in space vehicles. It was also thought that named craters should be at least 18 km in diameter. Conservatism and caution were stressed, the committee members pointing out that the names would be permanent, so only names of permanent renown should be used, revision and reassignment being thought undignified. Features below the minimum size were to be designated accordingly to the Mädler system, capital Roman letters being used for craters, valleys and other depressions, lower case Greek letters being used for hills, mountains and other eminences. In 1973 (*Trans. IAU*, **XVA,** 205; 1973) the working group went so far as to suggest that small features with diameters less than 100 m should have "given names" such as John, Mary and Nikolai, followed by the name of the nearby major feature. Scientists whose names were similar in sound to others and so omitted earlier were now to be given craters. So Born who had not been included because of his similarity to Bohr, Rutherford—Rutherfurd, Lawrence—Lorentz, were now in luck. In 1973 the qualification for a place in the nomenclature was also broadened but still the working group emphasised that they would "exclude all political figures, national heroes, religious figures, and modern philosophers. Ancient philosophers and various legendary figures will be accepted, however". The people responsible for Martian nomenclature (*Icarus*, **26,** 85; 1975) broadened their outlook still further bringing in biologists, geologists, atmospheric physicists and even science fiction writers concerned with the lore of Mars. The major sinuous channels were named after the name of Mars in a variety of other, largely non-IndoEuropean, languages.

Sagan advocates that the craters of Mercury should be named after great poets, authors, composers and other scholarly or artistic figures who were not physical scientists. He puts forward a large list of candidates containing such worthies as Dostoyevsky, Bertrand Russell, Picasso, Tschaikowsky and Hiram Bingham. The end result of the nomenclature should be a non-provincial distribution of nationalities, epochs and occupations; to quote Sagan "a distribution that our great-grandchildren can be proud of. It is just conceivable that some of them will be living in the places under discussion".

In the more specific cases of the satellites of Jupiter the IAU Working Group for planetary nomenclature have proposed a set of names for satellites V to XIII (*IAU Circular*, No. 2846; 1975). The Galilean Satellites are named after lovers or consorts of Zeus, the father of the gods in Greek mythology. The nomenclature group have tried to continue this tradition, naming the remaining satellites after friends of Zeus (all human, with the exception of Amalthea who was the goat which suckled the infant god). Sagan points out that the names proposed for VI, Himalia, and XII, Ananke don't fit the scheme, Ananke's being the mother of the Fates placed in judgment over the Olympian gods and not a friend of Zeus at all. Also Elara (VII), Lysithea (X), and Carme (XI) are thought, by Sagan, to be a touch obscure. He also finds it surprising that the most prominent remaining consorts seem to have been omitted and suggests that Maia, Leto, Semele, Demeter, Alcmene, Hera, Latona, Aegina, Pasiphae, Themis, Metis and Eurynome should be afforded due consideration.

The best chance for a place in the nomenclature for mere mortals, however, still remains the discovery of a comet or asteroid. □



100 years ago

Globigerina: from the 1872–76 voyage of the *Challenger*.
from *Nature*, June 1, **14,** 95; 1976.

Plant pathology changing direction?

from M. S. Wolfe and J. A. Barrett

A conference on the Genetic Basis of Epidemics in Agriculture was held in New York on April 5–8, 1976 under the auspices of the New York Academy of Sciences.

In 1970, disaster struck the American corn crop in the form of southern corn leaf blight, a fungal disease which reduced the total yield by 15% and thus rocked the world grain markets. In the same year, and for similar reasons, another cereal pathogen caused much greater suffering, but was less publicised: downy mildew of pearl millet in India reduced the yield of the "poor man's crop" by 30% and directly caused hunger and starvation. The similarity between the two disasters was the development of advanced forms of hybrid varieties which, in each crop, were dependent on a single type of male sterile cytoplasm and this provided the perfect uniform environment for the explosive development of the two pathogens.

One result of the corn epidemic was the instigation of an American survey of crop uniformity around the world, to determine the extent to which modern methods of agricultural production might be increasing the vulnerability of food production to pest and pathogen epidemics. Superficially at least, the result was alarming in that not only do the world's major crops comprise very few species, but that within each of these species production is dominated by very few varieties, which may often be related to each other.

The meeting was held to assess more critically the risk to world food supplies through the relationship between crop uniformity and disease and pest vulnerability, and the kinds of solutions which could be readily utilised. The general consensus was that since 1970, crop uniformity had, if anything, increased. In the developed countries, this was largely the result of demands by producer, processor and consumer for uniform products. In the developing countries, the main influence was the widespread introduction of breeding material from centralised sources which, at least in the recent past, has had a limited genetic base, and which squeezes indigenous material out of production, thus reducing available genetic resources.

In a comprehensive survey of the evolution of disease problems in tropical crops, I. Buddenhagen (International Institute for Tropical Agriculture, Ibadan) pointed out that increases in crop uniformity did not necessarily lead to problems, and that control in some cases could be effected by straightforward programmes of breeding for resistance. For example, downy mildew of sugar cane in Australia had been eliminated in this way, and in a similar way in Britain, it seems unlikely that eyespot disease of cereals will ever be a very serious threat because of the background of resistance built into cereal varieties grown in the United Kingdom.

For many diseases, however, notably those caused by the leaf pathogens of cereals, increased uniformity of crop production does place us on a knife edge with regard to disease and pest vulnerability. The problem was graphically illustrated by K. M. Safeeulla (University of Mysore) in relation to past and potential problems with the cereal varieties of the Green Revolution introduced into India. The core of the meeting was concerned with this problem and quickly revealed the wisdom and foresight of the organisers, P. R. Day, J. G. Horsfall and Otto Frankel, in bringing together those who are showing increasing interest in the genetics, epidemiology and mathematical interpretation of the dynamics of the host-parasite interaction as it occurs in the field. As with the early fusion of any range of scientific disciplines, opinions were many and varied, but two general trends could be discerned. First it was likely that there was no universal answer for dealing with all host–parasite problems: different strategies would be optimal for different situations depending on the balance between the level of disease risk and the price that plant breeders and growers are willing to pay to contain that risk. For example, the complete turn-away from crop uniformity by employing mixed varieties or multilines, which are heterogeneous for disease resistance and other characters, may be of advantage only where there is severe and continuous disease present. Second, there was a general, but not unanimous, movement towards the conclusion that no form of host resistance or its manifestation could produce an endlessly stable situation. The aim should be to slow down the rate of pathogen or pest response to the control measures so that it becomes virtually imperceptible, whilst maintaining constant vigilance.

The consideration that the host–parasite interaction could be stabilised, by the exploitation of so-called "horizontal resistance", was argued by R. Robinson (FAO, Rome). "Horizontal resistance" is defined as resistance in the host to all genotypes of the pathogen and Robinson suggested that techniques currently being used in an FAO programme under his direction could accumulate such resistance and thus provide permanent protection. Although some of the techniques have considerable practical value, we remained unconvinced by the theory, particularly since the weight of emerging experimental evidence moves us inexorably towards the view that specific qualitative or quantitative variation exists to corespond with all of the host resistances which have been thoroughly investigated. It does not necessarily follow that all of this variation will be of practical significance.

For all the tension which the pathologists and breeders feel with regard to the vulnerability of major crops to diseases and pests, and for all the differences of opinion experienced over the means of relieving that tension, there emerged from the meeting an underlying feeling of excitement and optimism. Plant pathologists have for too long been following behind the disasters, explaining why the patient suddenly became ill, or patching him up with pesticides. In recent years however, attention has been increasingly focused on positive strategies of confronting pathogens and pests with increasingly complex problems, based on sound genetical and epidemiological principles. The organisers of the meeting should feel well satisfied in providing an important signpost in this essential direction. □

Plant production in Pakistan

from D. Boulter and A. Muhammed

A Symposium on Genetic Control of Diversity in Plants was held in Lahore from March 1–6, 1976, organised by Dr Amir Muhammed, Vice-Chancellor, University of Agriculture, Lyallpur, and Dr A. Hollaender of the Associated Universities Inc., Washington, DC and sponsored chiefly by the US National Science Foundation.

THE initial promise of the introduction of new dwarf varieties of cereals and the impact of the Green Revolution have not been sustained in Pakistan; agricultural productivity remains disappointingly low. The Government of Pakistan recognises the first importance of agricultural improvement and has provided funds for buildings and facilities. The Punjab Finance Minister, Dr Abdul Khaliq, pointed out that Pakistan is "doomed", as he put it, to be an agricultural country; the only way out is by recognising the value of research which can bring about the revolutionary changes necessary to improve the general prosperity of the country. Thus, although primarily an agricultural country, only 35% of the gross national product derives from agriculture. Time is of the essence since every 5 minutes an acre of agricultural land is lost because of increased salinity. One major constraint on the improvement of agricultural productivity, is that Pakistani scientists lack channels of communication. Their work is often not recognised among their colleagues, and generally stimulation is lacking. So the Symposium was particularly welcome.

It was organised to provide a forum on the latest developments in the field of crop improvement, both fundamental and applied, including those relevant to the adverse conditions of high salinity and aridity.

O. H. Frankel (CSIRO, Canberra) warned of the drastic reduction in the diversity of crop plants brought about by widespread use of new varieties;

Pakistan with its gene centre of primitive wheats and fruits was an important case in point. The exploration and collection of genetic materials for future breeding programmes and the long-term conservation of seeds, are urgently required. S. A. Qureshi (Punjab Agricultural Research Institute, Lyallpur) made a strong case for the use of varieties developed from local programmes instead of selections from exotic materials, since the latter required inputs, particularly fertilisers, which were not being used effectively probably due to inadequate price incentives.

R. D. Brock (CSIRO, Canberra) stressed the important role of mutation breeding which is much underestimated at present; the best immediate prospects for increasing its specificity lie in the manipulation of the selection environment.

The development of high yielding, disease-resistant varieties of chick pea (*Cicer arietinum*) is an urgent need, since the supply of adequate protein still remains a problem in Pakistani diets especially for the young (A. Muhammed, University of Agriculture, Lyallpur). A. G. Kauser (University of Agriculture, Lyallpur) reported the successful development of reasonably high-yielding varieties with moderate resistance to wilt disease and high resistance to blight. C. M. Akhtar (Punjab Agricultural Research Institute, Lyallpur) reported varietal resistance to the major diseases of wheat, rice, sugarcane and cotton. Genetic resistance has not been observed against root rot of cotton, but biological control of this disease, by adding the antagonistic microorganism *Trichoderma viride*, along with organic substrate, has proved satisfactory but will need further field trials.

Soil salinity is the main problem in Pakistan especially where canal irrigation is practised, and at the moment the battle is being lost. Selection within hybrid rice populations for salt-tolerance appears feasible (M. Akbar, Nuclear Institute for Agriculture and Biology, Lyallpur) since crosses between salt-resistant varieties are more salt resistant than either of their parents and desirable progenies have been selected from F_4 populations. L. Bernstein (International Service Corps, New York) presented evidence that nutritional imbalance due to excess salt can often be corrected by foliar sprays of nutrients.

B. O. Eggum (Institute of Animal Science, Copenhagen) showed that up to 80% of the lysine in the wheat of some chappatis is made unavailable by cooking and that tannins, important constituents of tea, decrease the biological value of proteins. D. Boulter (University of Durham, UK) pointed out the great potential for improvement in the yield of legumes; the alternative strategy of increasing the protein content of cereals would be a difficult task due to the yield/protein content "trade-off". That higher protein content can be achieved without serious yield penalty, however, was illustrated by the results of V. A. Johnson (USDA, Nebraska) and his colleagues, who have recently released a new productive high protein hard winter wheat variety derived from Atlas 66, with genetic potential for 2 percentage points higher grain protein content.

J. Dobereiner (Universidade Federal Rural do Rio de Janeiro) outlined the potential for nitrogen fixation of a number of tropical forage grasses and sorghum, maize and wheat; she reported that the *Spirillum* bacterium responsible is found inside the roots. Exploitation of this potential requires considerable work to identify the limiting environmental factors and develop agronomically feasible practices to overcome them. The difference between cultivars and even ecotypes of some species has been demonstrated and preference should be given to plant breeding; for example, among S_1 lines of maize, several could be selected with nitrogenase activity many times higher than that of the original cultivars.

J. K. Setlow (Brookhaven National Laboratory, USA) looking to the future, thought that genetic transformation with purified DNA would be of limited value in agriculture, but that the use of restriction enzymes had great potential, in generating sites for the insertion of specific genetic material. □

Thermodynamics in biological systems

from a Correspondent

An EMBO-sponsored Workshop on Thermodynamics in Biological Systems was held at the Villa Durazzo at Santa Margherita Ligure under the auspices of the University of Genoa on April 5–9, 1976.

The contributions of thermodynamics in general, and calorimetry in particular, to the study of biochemical and biological processes formed the subject of the workshop.

S. N. Timasheff (Brandeis University) presented a thermodynamic analysis of the aggregation of tubulin which, in the presence of Mg^{2+} at low temperatures, yields cyclic polymers (molecular weight 3×10^6) composed of 26 subunits. Above 37 °C the aggregation to microtubules (molecular weight $\sim 10^8$) is favoured, particularly in the presence of glycerol or sucrose. The thermodynamic and sedimentation data were confirmed by electron micrographs showing the different types of aggregates. A similar treatment, applied to the aggregation of sickle cell haemoglobin, was discussed by P. Ross (National Institutes of Health, Washington), on the basis of the polymerisation of critical nuclei, consisting of about 40 molecules, to helical structures.

With the aid of selective chemical modification of Trp 62 or Glu 36, J. Rupley (University of Arizona) demonstrated the involvement of these residues in the self-association of lysozyme. In the case of insulin, the three dimer-hexamer association characteristic of the crystal state is inconsistent with calorimetric, scattering and sedimentation studies in solution.

The thermodynamics of small molecule binding to biopolymers provided considerable interest. Thus H. J. Hinz (University of Regensburg) showed that whereas isoleucine tRNA synthetase showed a high affinity for isoleucine, compared with leucine or valine, yet ΔH and ΔCp for the binding of all three amino acids are identical, suggesting COO^- and NH_3^+ groups as the interaction sites. M. N. Jones (University of Manchester) reported on the binding of sodium dodecyl sulphate to β-lactoglobulin which indicated two specific, high affinity sites ($\Delta H = -9.5$ kJ mol^{-1}) and 22 non-specific sites ($\Delta H = -3.5$ kJ mol^{-1}). In this context, but on a much more complex level, A. Beezer (Chelsea College) described applications of microcalorimetry to characterise interactions of micro-organisms with drugs. Interpretation of such experiments are frequently complicated by specific interactions with certain buffer ions.

High precision heat capacity measurements on biopolymers over wide temperature ranges (5–500 K) continue to improve our understanding of the unfolding mechanisms. P. L. Privalov (Institute of Protein Research, Moscow) reported on methods of resolving $Cp(T)$ curves from various types of tRNA into several Gaussians which are found to correspond well with those derived from NMR measurements. The existence of "stable" intermediate states is therefore a possibility and the minimum domain size is estimated as about 100 residues. Comprehensive thermodynamic state diagrams, that is, free energies, enthalpies and entropies as functions of pH and temperature for both native and unfolded states, were presented by W. Pfeil (Zentralinstitut für Molekularbiologie, Berlin) for myoglobin, cytochrome *c*, haemoglobin, chymotrypsin and lysozyme. Such diagrams can now provide a thermodynamic base of reference for "unfolded" proteins. J. Brandts (University of Massachusetts) discussed the unfolding of ribonuclease in terms of the possible intermediate states which are clearly indicated by kinetic measurements, although the thermodynamics are compatible with a two-state model, implying that all steps bar one are associated with zero enthalpy changes. This condition is met by the *cis-trans* isomerisation of proline which may therefore account for the rate-determining step, whereas conformational changes are associated with relaxation times of 1–100 ms. The folding/unfolding equilibria of all small proteins appear to be consistent with a rate-determining step corresponding to a *cis-trans* isomerisation.

A description of the dynamic nature of folded proteins presents many topical problems and both R. Lumry and A. Rosenberg (University of Minnesota) stressed the importance of high frequency ($10^8\ \mathrm{s}^{-1}$) conformational fluctuations to the catalytic functions of proteins. The existence of free volume fluctuations is consistent with the high diffusion rates of large molecules (such as acrylonitrile) in folded proteins and with the marked pressure dependence of proton exchange rates which are not observed with homopolypeptides or oxidised ribonuclease.

A thermodynamic characterisation of biochemical processes by itself is not always as interesting as an insight into molecular mechanisms, and this implicitly takes us into the realm of statistical thermodynamics. F. Franks (Unilever Research, Sharnbrook) discussed the combination of thermodynamic data and computational methods aimed at the derivation of a potential function for the hydrophobic interaction. This in turn allows the calculation of the radial distribution function and hence the thermodynamic properties associated with the interaction of apolar particles in water. In a complementary paper, E. Clementi (Istituto Donegani, Novara) outlined a combination of *ab initio* and computer simulation approaches to the study of amino acid hydration. □

Exploding black holes

from P. C. W. Davies

A symposium organised by R. Penrose and D. Sciama was held in Oxford on April 20 and 21, 1976.

BLACK holes are either a French, German or American idea, according to opinion. But if they explode, they are British. At an historic Oxford symposium early in 1974, Stephen Hawking of the University of Cambridge, revealed an astonishing theoretical discovery—black holes are not black after all, but radiate with a characteristic blackbody (thermal equilibrium) spectrum, at a temperature which increases as the mass dwindles, ensuring the eventual demise of these objects in a sudden explosion.

Since then, the analysis of what is now referred to as the Hawking process has become a veritable industry in South East England, with no less than three major relativity groups (Cambridge, London and Oxford) actively studying the details and implications of the new discovery. Hawking's prediction itself was the result of applying quantum theory to black holes. The remarkable conclusion is that the violent distortion of space-time in their vicinity leads to the production of radiation with a definite temperature. The significance of this lies not so much in observation (the temperature of a solar mass black hole is a mere 10^{-6} K) but in establishing important theoretical connections hitherto barely suspected.

It had previously been noted by some relativists that black hole processes bear a superficial resemblance to thermodynamics. As long as the treatment remained classical the resemblance was only an analogy. The key step, which Hawking took, was to apply quantum mechanics. The result has been to provide an amazing underlying connection between quantum theory, general relativity and thermodynamics.

As experts in any of these three disciplines tend to be lamentably ignorant of the other two, it was particularly appropriate that this year's Oxford symposium entailed a programme of lectures and reviews by practitioners of each subject. The conference provided an opportunity for an unusual interplay of ideas, with the Hawking process as a central theme.

Hawking himself delivered some provocative and fascinating extensions of his original idea, by dealing with the conceptual and philosophical aspects of particle production effects. He and G. Gibbons (University of Cambridge) presented arguments in support of the proposal that event horizons cause radiation production under quite general circumstances. In particular, the claim that particle detectors in de Sitter space would also detect radiation seems to be a direct challenge to the conventional understanding of some fundamental aspects of relativity and quantum theory. This challenge was taken up by some of the other participants in fruitful but inconclusive exchanges. Controversy centred around Hawking's claim that much of physics is observer-dependent. Two observers in different states of motion might encounter two entirely dissimilar histories of the Universe, he alleges. In particular, the traditional theoretical procedure of attempting to construct a covariant energy-momentum tensor which is independent of observer is misguided. Ironically, this procedure is precisely what several research groups have been trying to do, with a view to studying how quantum effects react back on the geometry of space-time. According to Hawking, the traditional energy-momentum tensor calculations are wrong. Indeed, they contain so-called vacuum polarisation terms, which he said he did not believe in.

It will take some time for the full implications of these intriguing new ideas to sink in. The more traditional approach was defended by Bryce DeWitt (at present at the University of Oxford). He described some of the conventional energy-momentum tensor calculations, and also presented an interesting model of a particle detector which appeared to conflict with Hawking's radical departure.

Much of the remainder of the conference was devoted to exposition about some of the peripheral aspects of the Hawking process. Some of the review talks were interesting and entertaining in their own right, particularly that of Donald Lyden-Bell (University of Cambridge) who presented a brilliant review of the thermodynamics of classical self-gravitating systems. Other speakers, such as Jergen Ehlers (Munich) who spoke about relativistic kinetic theory and statistical mechanics, and Bernard Schutz (Cardiff), who dealt with stability in relativity, clearly touched on subject matter which will eventually be relevant to the hoped-for unification of quantum field theory, thermodynamics and gravitation. But at this stage only a jumble of half-connected ideas has emerged. Other speakers were John Steward (Cambridge), Robert Thouless (Birmingham) and Oliver Penrose (Open University).

The cross-fertilisation between these subject areas is clearly of great importance in stimulating further research in this new subject. The conference organisers, Roger Penrose and Dennis Sciama, both gave thought-provoking general assessments, tying the strands of the separate topics together. It is hard to resist their own impression that much more lies beneath the surface; many disconnected ideas seem to be associated in some, as yet vague, but intriguing fashion. In this fast-moving field of research, it is probable that another Oxford symposium in the near future will provide still more fundamentally new results, which could well have implications far beyond the specialist topic of black holes. □

Designed Etched & Published by George Cruikshank
Myddelton Terrace Pentonville June 25th 1836
Giraffes — Granny-Dears, & other Novelties

Mary Evans Picture Library

The Zoo: 150 years of research

L. Harrison Matthews*

In the 150 years since its inception, the Zoological Society has developed from a collection of curiosities on which only observational research was carried out, to one of the world's most comprehensive collections of animal species, with two attached experimental research institutes. Dr Harrison Matthews traces the evolution of the Zoo from the time when the idea first occurred to Sir Stamford Raffles on a visit to the Jardin des Plantes in Paris.

IN the first quarter of the nineteenth century, towards the end of which the Zoological Society of London was founded, the science of zoology was emerging from the collecting of objects of natural history for the cabinets of the curious to become a discipline as scientific as botany and deserving the attention of people more serious than dilettanti. The works of Cuvier on the relationships of animals stimulated interest in the study of morphology among many naturalists who followed his researches on comparative anatomy and palaeontology. They broke away from the linear concept of classification built on the foundations laid by Linnaeus, who was the first to lecture in a university on zoology and botany as subjects in their own right instead of as adjuncts to medical knowledge. The immense numbers of hitherto unknown animals brought to the notice of European naturalists by the exploration and settlement of distant parts of the world gave descriptive zoology work enough to occupy many in describing and classifying the novelties.

Sir Stamford Raffles, who spent most of his short life in the service of the East India Company, and who was Lieutenant Governor first of Java and later of Sumatra, and was the founder of Singapore, gained a vast knowledge of the plants and animals of the Far East, in particular the natural history of Java, Sumatra and the Malay Archipelago. In 1817, after leaving Java and before going to Sumatra, he was in Europe, and visited Paris where he was greatly impressed by the Jardin des Plantes. Later in the year he returned to England, where he was knighted and published his *History of Java;* but more important for the present subject he discussed with Sir Joseph Banks, then President of the Royal Society, a proposal inspired by what he had seen in Paris for the foundation of a zoological society in England, to be run on similar lines to the Jardin des Plantes.

Although Banks died in 1820 the proposal was kept alive by correspondence with Sir Humphry Davy and others after Raffles went to Sumatra in 1817, so that on his final return to England in 1824 he became chairman of a committee to consider the formation of the society. The committee's prospectus gave the objects of the society as "the introduction of new varieties, breeds, and races of animals for the purpose of domestication or for stocking our farm-yards, woods, pleasure-grounds, and wastes with the establishment of a general Zoological Collection, consisting of prepared specimens in the different classes and orders, so as to . . . point out the analogies between the animals already domesticated, and those which are similar in character upon

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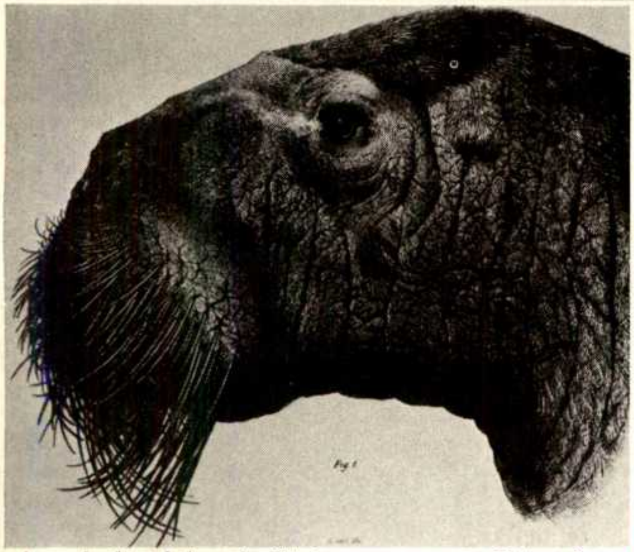
*The Old Rectory, Stansfield, Suffolk



A male bearded seal, *Trichecus rosmarus*. Taken from volumes of the *Transactions* of the Zoological Society.

Zoological Society

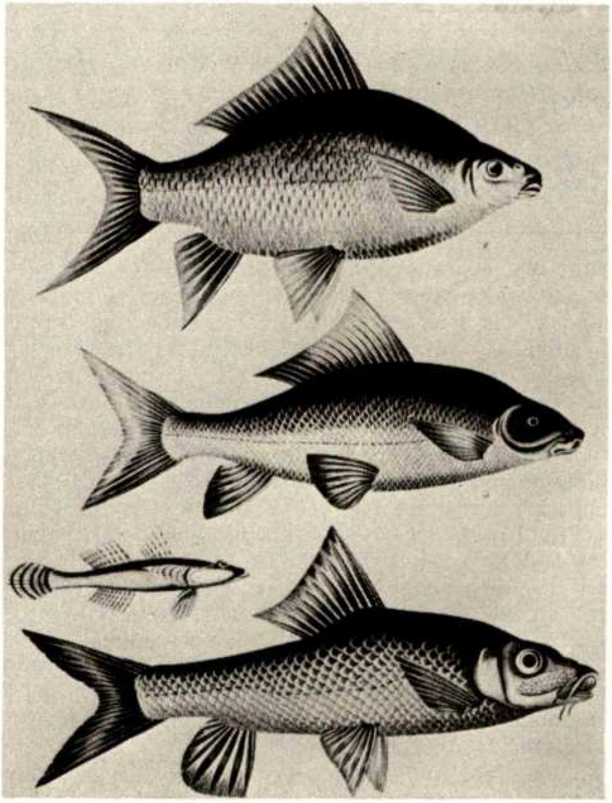
which the first experiments may be made."

After long negotiations and the granting by the government of a site in Regent's Park the Zoological Society was founded in 1826 for the advancement of zoological knowledge and "the formation of a Collection of living Animals; a Museum of preserved Animals; and a Library connected with the Subject", with Raffles as president. Two months later Raffles died suddenly, and in the following year his widow gave the Society's museum his fine collection made in Sumatra; some of it went to the British Museum when the Society dispersed its museum in 1855. In 1829 the Society was incorporated by Royal Charter as "The Zoological Society of London" for the advancement of zoology and animal physiology, and the introduction of new and curious subjects of the animal kingdom—'animal physiology' had not then acquired its present meaning, but covered the feeding, breeding, and acclimatisation of animals. Raffles acknowledged that he himself was more interested in the scientific side whereas Sir Humphry Davy looked more "to the practical and immediate utility to the country gentlemen".

Raffles and Davy enlisted the help of the Aristocracy and Church in founding the Society so that the list of the first committee was full of "top brass" with a tail chiefly of amateur zoologists: in 1929 Chalmers Mitchell, then secretary, wrote that "from the beginning, the Society depended very slightly on the support of professional zoologists, and largely on persons of public importance—a tradition that it has always maintained." The Society's gardens were opened early in 1828, and the normal course of mortality soon began to supply specimens for examination and dissection by the anatomists, or for preservation in the Museum. The type specimens of newly described creatures were stuffed when they died and displayed in the cases; they were given to the British Museum when the collection was discarded but the rest of the contents was sold, some to the British Museum and some to various provincial museums.

Although a lecture was delivered to a general meeting of the Society in 1827 by Joshua Brookes, the proprietor of a private anatomy school, on the dissection of the body of an ostrich which had been given to the Society by George IV after it died at Windsor, scientific meetings did not start until 1830. At the first meeting for scientific business held in the autumn of that year the secretary, N. A. Vigors, an enthusiastic ornithologist, read a paper on the crested quails of America describing and naming two hitherto unknown species. He was followed by Richard Owen who read a paper on the anatomy of an orang-utan that had died shortly after reaching the Society's collection and had been sent to him for dissection. These communications set the pattern followed by scientific meetings for nearly a century, concentrating for the greater part on systematics, faunas, the description of new species, and on morphology and anatomy.

In the 1830s a visiting veterinary surgeon treated sick animals and made occasional post-mortem examinations to find the cause of death in some of the animals that died, but the bodies of dead animals were generally given to such anatomists as were interested, for dissection and study in their own laboratories. It was not until 1865 that Professor T. H. Huxley spurred the Society into providing a dead-house and dissecting room, and to appointing a "prosector" in charge of them. This title was a misnomer, because a prosector is an assistant who prepares demonstration dissections for the lectures of a professor; the name was, however, retained for many years. The duties of the post combined those of pathologist and research anatomist, and the distribution of material to museums and academic workers who had asked for it to be supplied when available. For some sixty years the successive holders of the post gave greater attention to pathology or anatomy as they preferred—a large number of anatomical memoirs was produced by the earlier prosectors. In 1903 a pathologist as well as a prosector was appointed and, after a number of short term or part time workers had held the post, the Society in 1924 joined with the London School of Tropical Medicine and Hygiene, whose new buildings were not yet ready for occupation, in financing a Research Fellowship in Comparative Pathology, to the great advantage of the



From top to bottom: *Cyprinus abramioides*, *Varicorhinus babree*, *Gobus kurpah* and *Barbus mussallah*. Taken from volumes of the *Transactions* of the Zoological Society.

Zoological Society

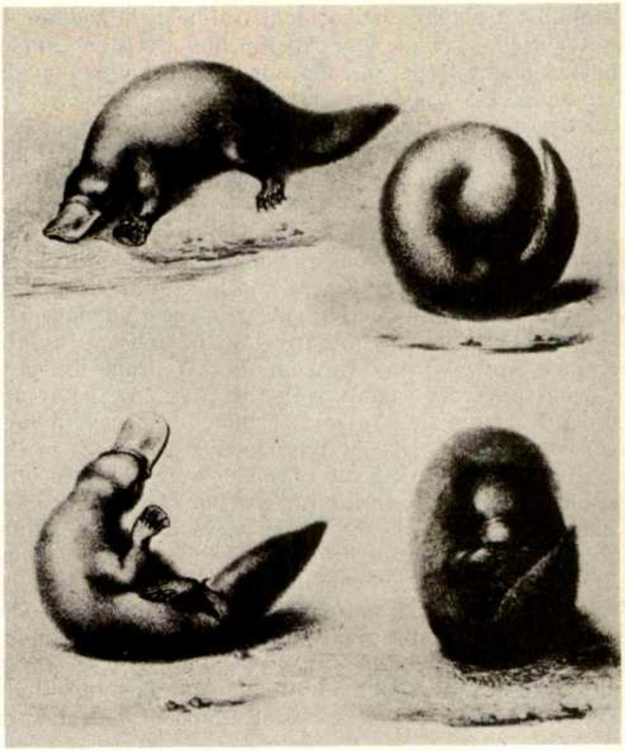
work on the Society's material. This arrangement lasted until 1928, when the Society appointed its own full-time pathologist, a post that continues today. In 1925 the post of prosector was abolished and replaced by a part-time research fellowship—though the title was revived later for a short time. Thus for nearly the first hundred years of the Society's existence the research carried out on its own premises was confined almost entirely to the descriptive anatomy of animals that died in the collection, some descriptive pathology, and the occasional description of new species from animals living in the gardens.

From the first, in 1830, the proceedings of the scientific meetings were published as a journal, which soon became the familiar "P.Z.S.", the *Proc. zool. Soc. Lond.* of reference lists. In its early years it was indeed a report of the proceedings, but as time went on it took the usual form of a scientific journal containing formal papers, some of which had been read in full at the scientific meetings, others only in summary or title, few of them based on work done in the gardens. The title was retained until 1965 when it was changed to the more appropriate *Journal of Zoology*—with a few sentimental sighs from some. In 1833 a second publication appeared, the *Transactions* of the Society, quarto in size to take papers that needed large illustrations; it was issued in parts at irregular intervals. The illustrations, especially those prepared in the last century, are superb, some of them very large folding plates, most beautifully lithographed and often hand coloured. Those of anatomical dissections and of bones, whether fossil or modern, are no less works of art than the illustrations of bright plumaged birds and of butterflies or other insects. Similar plates on a smaller scale were also often used in the *Proceedings.* The cost of producing these publications was great, and until recent decades was subsidised from the general funds of the Society, which thereby fulfilled one of its charter aims, of advancing zoology, in a very material way. With the fall in the value of money this is no longer possible, and the publications have to be financially self-supporting.

The publications were exchanged from their start for those of other learned societies all over the world so that part of their cost was recovered in kind to lay the foundation of the Society's magnificent zoological library. This is one of the largest and most comprehensive in the world, now housed in new premises with an excellent reading room and skilled staff appropriate to the importance and value of such a collection, to which all workers engaged in zoological research can have access.

From time to time the Society made grants towards the expenses of traveller-naturalists, generally with the stipulation that it should receive the living animals collected. Such collecting trips certainly advanced zoology in faunistic knowledge, but scarcely came into the category of academic research.

Apart from its publications the Society's support for research was thus mainly confined to observations on dead animals until the 1930s, when J. S. Huxley became Secretary. He was an early worker on animal behaviour following in the footsteps of Edmund Selous, who invented bird-watching in 1901; his well known paper on the courtship of the great crested grebe was published by the Society in 1914. In the few years before the outbreak of war in 1939 he himself studied animal behaviour on animals living in the Society's gardens, and helped others, among them James Fisher, in similar work. This trend towards the study of living animals is shown in the choice of subject by one of the early holders of the Research Fellowship, the present Secretary, who was appointed to the post as a young man shortly before Huxley joined the staff. Dr Zuckkerman, as he then was, observed and analysed the behaviour of the colony of Hamadyras baboons that made "Monkey Hill" one of the sights of London, approaching the subject "from the deterministic point of view of the physiologist", although he is described as "Anatomist to the Society" on the title page of the book in which he records and discussed his researches. This was what would now be called a seminal work, though its conclusions have been modified in the light of work on animals in less unnatural surroundings and in populations of more normal sex ratio.



Zoological Society

Duckbilled Platypus, *Ornithorhyncus paradoxus.* Taken from volumes of the *Transactions* of the Zoological Society.

The outbreak of war in 1939 put an end to this new approach to research: by 1945 a long list of dilapidations had accumulated, so that neglected repairs, the restoration of bomb-damage, and replenishing the collections took most of the Society's current resources until the 1950s when the present Secretary, now Lord Zuckerman, was appointed. The old sanatorium, and the prosectorium which combined dissecting-room with pathology laboratory, were quite inadequate for any serious work, though they had served well enough in their day. A new and well equipped animal hospital, and modern pathology laboratories were built, a full time veterinary officer and a new pathologist were appointed with a supporting staff of qualified technicians. When vacancies occurred new curators were appointed to the various departments, so that they are now all academically qualified zoologists. A steady stream of research publications has flowed from the work of these officers and from visiting workers in their departments.

The most impressive developments in research, however, are the establishment of two research institutes in the Society's gardens, with splendidly equipped modern laboratories, animal rooms, and ancillary services. The Nuffield Institute of Comparative Medicine, and the Wellcome Institute of Comparative Physiology owe their conception to the foresight of Lord Zuckerman, and their realisation to his genius for fund-raising and to the generosity of several philanthropic foundations and trusts. The work undertaken by the institutes differs fundamentally from the researches formerly sponsored by the Society in being experimental and not solely observational. The latter were necessarily restricted to work that does not need a Home Office licence under the Cruelty to Animals Act, because considerations of public relations and sentiment, in addition to the Act itself, preclude any experimental work on animals exhibited in the Society's collection: the institutes, on the other hand, are licensed. The latest available reports on the work of the Institutes show the wide range of subjects covered, many of them of immediate value in preserving the health and well-being of the collections. In the Nuffield Institute of Comparative Medicine the departments for pathology, infectious diseases, biochemistry, and radiology have investigated numerous problems arising in the collections, as well as more general topics with application to human as well as veterinary medicine. The Radiology Department also maintains the growing and much consulted Wellcome Animal X-ray Museum. The Wellcome Institute has been concerned mainly with the physiology of reproduction in mammals. An investigation of special interest has been carried out on the reproductive physiology of the hystricomorph rodents, some of which were collected during visits to South America for the purpose by members of the staff. Several of the species have bred successfully in the Institute. Other studies include work on the viability of spermatozoa, on the possibility of artificially inseminating African elephants, and reproduction in the Mustelidae. The Institute also contains a growing reference collection of reproductive and endocrine organs.

The Institutes give hospitality to a steady flow of visiting workers, and send members of staff abroad for field work or to visit other institutions, with some of which they have special affiliations. The activity of the Institutes is illustrated by the long list of scientific papers published each year by members of staff and visitors, in the *Journal of Zoology* and the publications of other learned societies.

Since 1959 the Society has held several symposia every year on special topics to bring together workers engaged in research on different aspects of any branch of zoology. The papers and discussions are printed in a special series of Symposia publications which has a wide circulation. The *International Zoo Yearbook,* published annually since 1960 is addressed mainly to those concerned with the housing and husbandry of animals in captivity, but it does contain accounts of research pertinent to those subjects.

Finally, an important publication of long standing must be mentioned. The *Zoological Record,* issued annually since 1864, is an essential tool to all engaged in research on zoology; it is an international bibliography listing all papers published in all branches of zoology for each year, with author, subject, and systematic indexes. The enormous growth in the literature during the past twenty years is reflected in the size of the annual volume, now prepared by a whole-time staff of specialist recorders.

The shades of Raffles and Humphry Davy, could they glimpse the developments at Regent's Park as they stroll through the groves of the Elysian Fields, could surely be surprised to see what they had started, and would be gratified to know that their ideas for the advancement of zoology are being so energetically realised in the researches made possible by their Society.

articles

Isotopic tree thermometers

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Evidence is summarised here that trees store a record of atmospheric temperature in their rings. In each ring, the ratios of the stable isotopes of hydrogen and oxygen vary with the air temperature prevailing when the ring was formed. We have shown that the temperature records in three modern trees seem to follow the local mercury thermometer records, and have found that a Japanese cedar indicates a temperature fall of ~1.5 °C in the past 1,800 yr.

LONG TERM isotope changes in precipitation, caused by changes in climatic temperatures, are well documented in polar ice caps —the heavier of the stable isotopes is depleted in ice laid down in the ice age by comparison with present-day ice. In 1970 we extended this concept to trees, suggesting that they are also thermometers, and undertook measurements to test the idea. In trees which use atmospheric precipitation to manufacture their rings, isotope variations in the rings should be climate indicators because the isotope composition in the rain and CO_2 varies with temperature.

Many tree ring sequences can be counted with an accuracy of about one year. Those which are not yet tied to modern sequences by overlapping ring patterns (said to be "floating"), can be dated in favourable cases with an accuracy of about 30 yr, by radiocarbon dating, depending on the age and on the number of radiocarbon measurements which are made. The isotopic compositions of atmospheric carbon dioxide, rain and snow have been shown to vary strongly with atmospheric temperature[1,2]. A non-negligible temperature-dependent isotope fractionation in the formation of wood has been computed assuming thermodynamic equilibrium but is small by comparison with that expected in rainwater[3]. Therefore, the tree ring emerging from the outer living layer of sapwood should record the average temperature of the atmosphere at that time, except for possible isotope exchange between the heartwood and water. The matter of isotope exchange between intact heartwood and water does not seem to be a serious threat since it has been shown, at least in species having tight rings, that the sap does not interact with the heartwood. Huber has shown, using biological dyes in many tree species, that water conduction remains limited to the outermost annual ring[4]. In addition, radiocarbon injected into or ingested by a living tree does not exchange with neighbouring rings[5,6]. Furthermore, we have observed that when *Sequoia gigantea* wood was soaked for 37 d in an air atmosphere saturated at 100% humidity, and previously labelled to $\delta D_{(\text{SMOW})} = 1{,}170‰$, no deuterium exchange was detected even though water saturation was optically evident. This is reasonable because wood is a most remarkable polymer, containing very large molecules, largely cross linked internally and to each other with hydrogen bonds. Furthermore, the bonds of cellulose and lignin are very strong, by comparison with almost any other molecular bonds, so that isotope exchange at room temperature with water or dissolved CO_2 becomes very improbable.

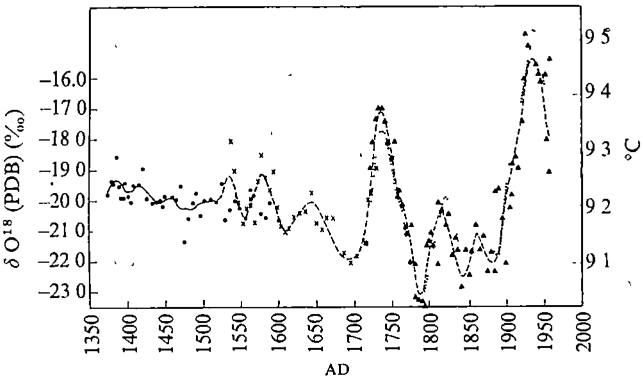


Fig. 1 ^{18}O ratio in German Oak, *Quercus petraea* (Spessart Mountains), compared with the annual average temperature of England (. . .). Thirty-year running averages of each isotope ratio are plotted against tree ring years. Five-sample (or ~ 30 yr) running average for oak from Marburg, Germany; ●, 4.7-yr sample; ▲, Seven-sample (~ 30 yr) running average for Spessart oak No. 1; × five-sample (~ 30 yr) running average for Spessart oak No. 2. The temperature record of England is shown here for comparison because it extends much further back than do the next most lengthy records, namely those for Basel and Geneva. The agreement with these latter temperature records is equally good, however. PDB, Pee Dee Belemnite, a fossil shell used as a standard; ———, smoothed by eye.

Temperature records

The trees most useful for testing this method of determining temperature histories and making calibrations are European trees since only Europe has temperature records extending back three centuries[7] (for England) and more than two centuries (for Basel and Geneva).

To demonstrate the effect experimentally, we needed to measure modern trees whose rings have been correctly counted and to obtain lengthy mercury temperature records from places near where they grew, preferably from the same altitude. The oldest temperature records in the World Weather Records are for Basel and Geneva, at about 300 m. We therefore obtained from the laboratory of B. Huber in Munich (the only tree ring laboratory in Europe when we started this work) a German oak, grown in middle Germany at an altitude of about 300 m, which had been correlated with the fiducial tree ring sequential pattern developed by Huber and his colleagues[8]. Later we obtained samples of similarly calibrated trees from his students, B.B. and D. Eckstein.

So far, we have measured isotope ratios in three modern trees of three different species and have compared them with local temperature records from mercury thermometers; we have also studied two old trees of two different species and compared the isotope ratios with temperature records deduced from surrogate evidence such as lateness of cherry tree bloomings, number of snow days per year, and number of days per year when lakes were frozen. We found significant correlations.

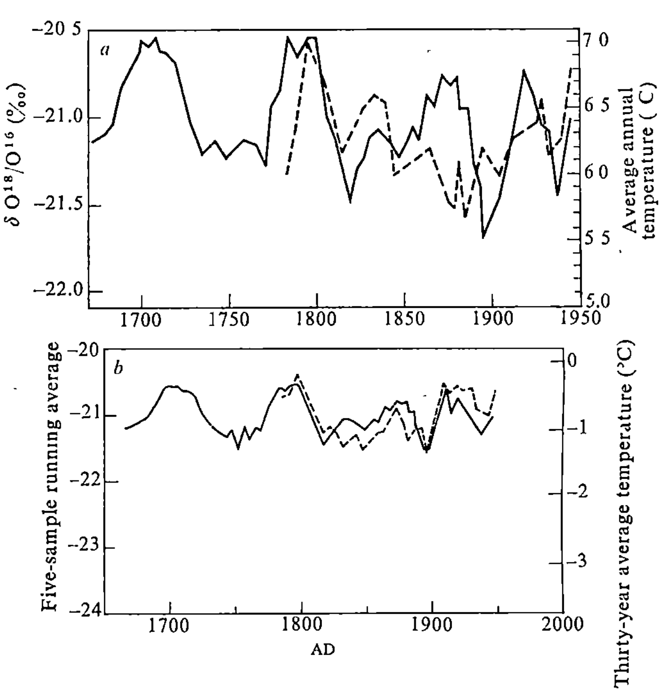


Fig. 2 ^{18}O ratio (—) for Bavarian fir, *Abies alba*, compared with: *a*, annual average temperature; *b*, average of January, February and March temperature for Höhen Peissenberg, Bavaria, at the same altitude of about 1,000 m above sea level, but about 150 miles south-west of the Bavarian Forest where the fir grew. This is mountainous country so that local differences in temperature may be expected. Temperatures for January, February and March seem to fit as well or better in all three modern trees analysed so far. This might perhaps be explained by the fact that the trees we have analysed up to now have grown where winter snow collects and melt water freezes into the ground. When the ground thaws, sap begins to flow (for example, the voluminous flow of maple sap and turpentine sap in early spring) and growth begins (for example, the fast rate at which pines shoot their candles in early spring). In the past three centuries, judging from mercury thermometer records, the major climatic change has been in temperatures during winter months. Temperatures in summer months have remained relatively constant since thermometer records began.

In Fig. 1 we compare ^{18}O measurements for a German oak, *Quercus petraea*, the rings of which were counted by Huber and Siebenlist[8], with English temperature records[9-11] back to the time of the invention of the thermometer in the late seventeenth century. The temperature coefficients have been evaluated by least squares analysis[12,13]. Figure 2 shows the ^{18}O data for a Bavarian fir, *Abies alba*, the rings of which were counted by Becker and Siebenlist[14]; they are compared with temperature records made near where the tree grew[7]. Similar results have been obtained with deuterium measurements (Fig. 3) for the oak.

General applicability

Since the method seems to have worked for European oak and fir, we felt that it would probably work elsewhere. Direct measurements of the isotopic composition of atmospheric moisture (as rain and snow) and indirectly of carbon dioxide, the ^{18}O of which is probably in isotopic equilibrium with atmospheric moisture[15], have been made and are available in the publications of the International Atomic Energy Agency (IAEA)[2]. Figure 4 shows the ^{18}O and D correlation with temperature for Austrian stations for the past 15 yr or so. The isotope composition of precipitation varies similarly with

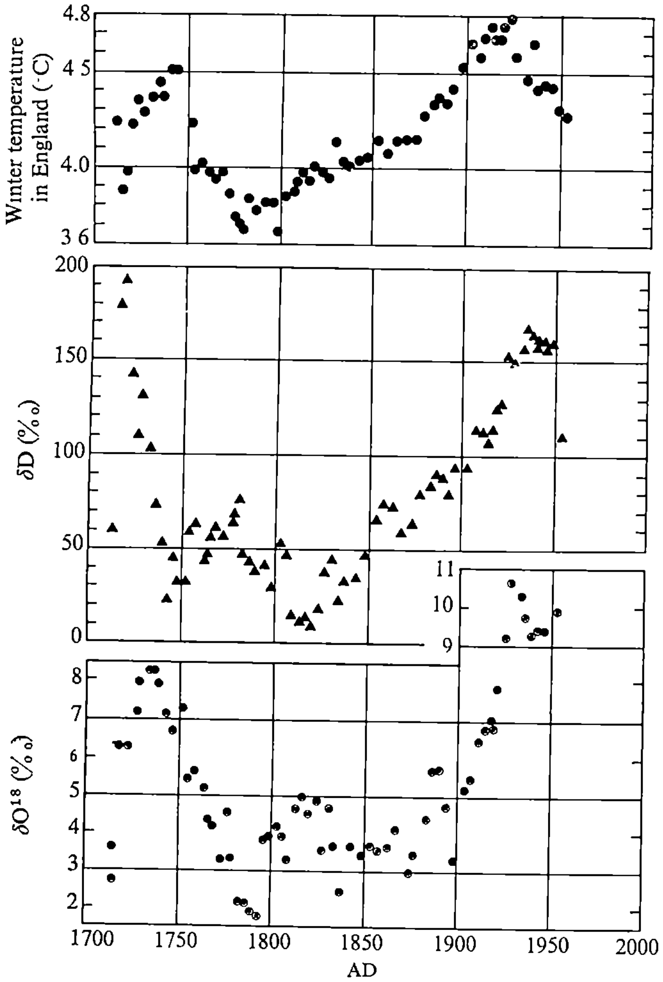


Fig. 3 Deuterium and oxygen ratios in German oak compared with English winter temperatures (30-yr running averages, see ref. 11). The procedure we used was to sample with a milling tool a number of tree rings, obtaining about 5 mg per sample; heat with $HgCl_2$ to produce CO_2 and HCl (ref. 22); remove the HCl with quinoline; and measure the ^{18}O abundance in a mass spectrometer. For measurement of the deuterium, we burnt the wood, reacted the water with metallic uranium to produce hydrogen gas and measured its D abundance in a hydrogen mass spectrometer (negative ratios).

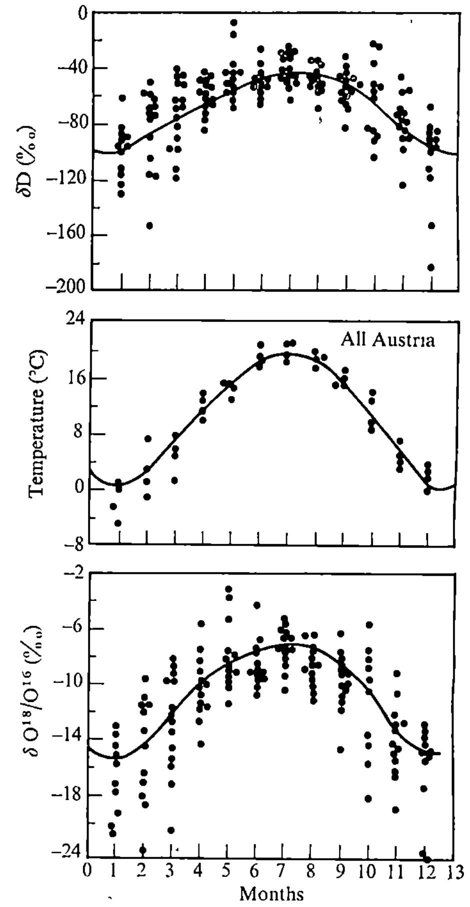
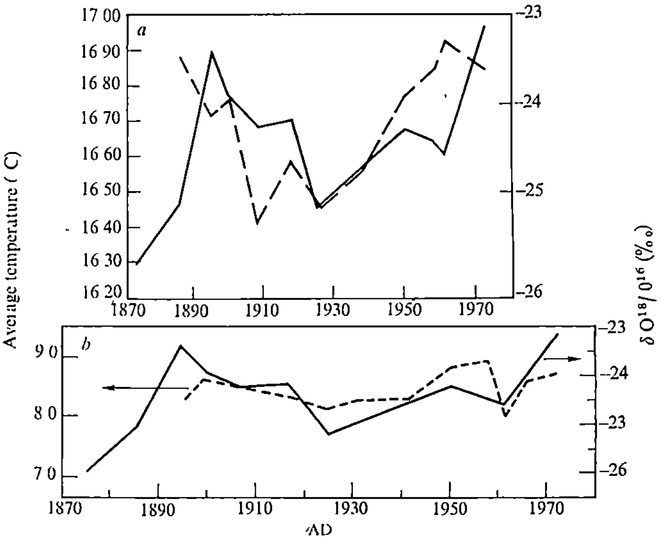


Fig. 4 Deuterium and ^{18}O isotope measurements in Austrian precipitation (Vienna 1961–71, Petzenkirchen 1966–71, Apetlon 1966–69, Podersdorf 1966–68) compared with average monthly temperature (Vienna 1961–64) taken from ref. 2. We have also plotted the IAEA data from these years for Stuttgart and many other places and find similar temperature dependences. When the climate deteriorates or improves, presumably the curve of annual dependence moves down or up on the isotope axis; the IAEA data have been measured for only about 20 yr, so long term changes have not yet been demonstrated.

Fig. 5 ^{18}O (—) in modern *Cryptomeria japonica* compared with: *a*, average annual; *b*, January, February and March air temperatures measured at Miyazaki, Japan[19]. The *Cryptomeria* grew at an altitude of 1,350 m, whereas Miyazaki is at sea level, about 150 miles north-east of Yaku Island. Averages over about 8 yr are shown.



temperature in many other places, as may be seen by plotting the IAEA isotope data against air temperature[2].

Old Europe and Japan

Thus we have had the temerity to measure German oak rings which grew before thermometers were available[8]. In Fig. 1 we show warm intervals at AD 1530, 1580 and 1650 and cold periods at about AD 1700 and 1800, in agreement with Bergthorsson's deductions of climate variations in Iceland[16], and in agreement with the historical evidence of severe climate deterioration in the First and Second Little Ice Ages in Europe.

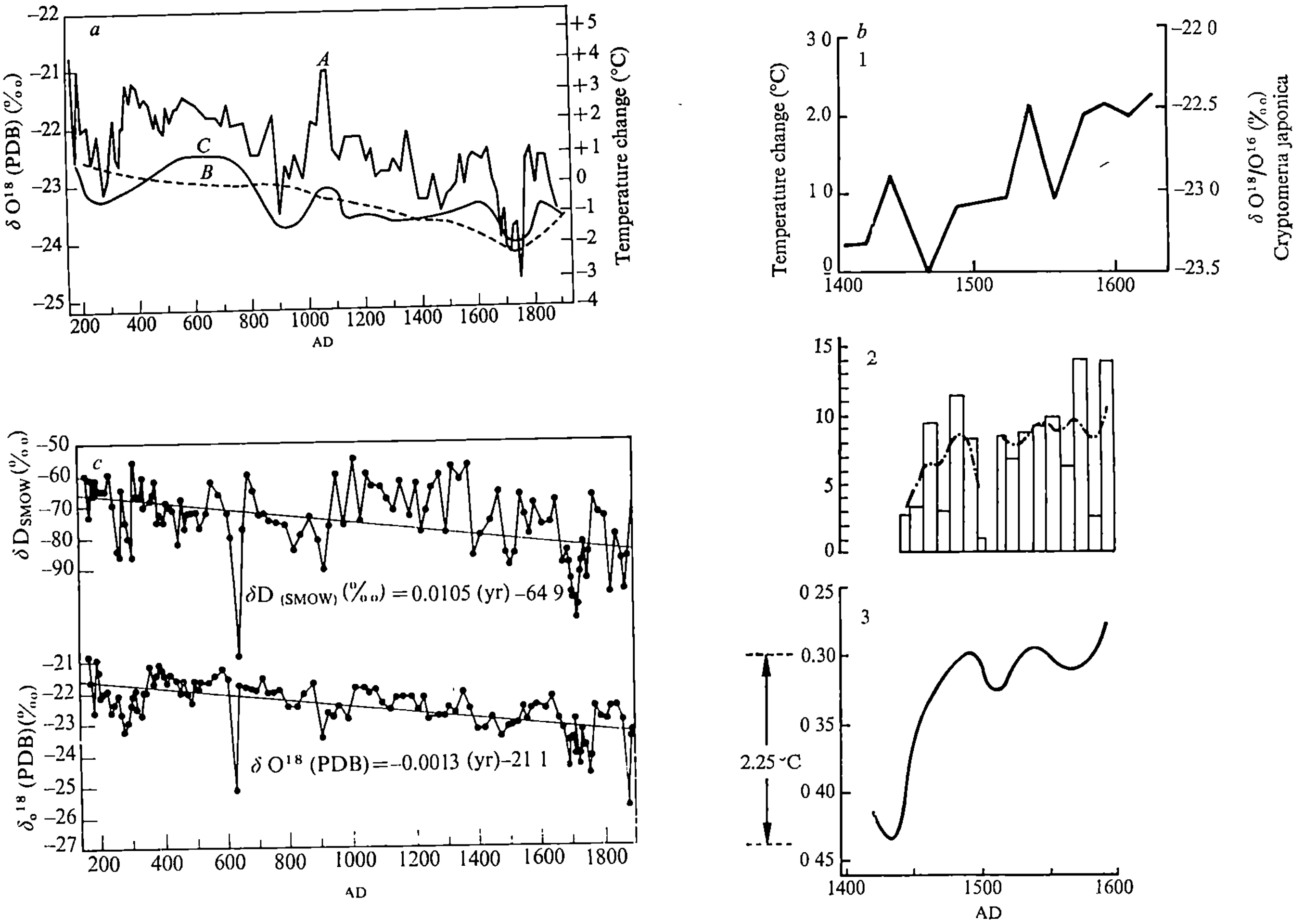


Fig. 6 ^{18}O ratio (*a*) in historic *Cryptomeria japonica*: *a*, compared with Chinese air temperatures (*b*) and Norwegian snow line (*c*)[19]; *b*, compared with Japanese air temperatures[18]. Averages over about 8 yr are shown. *b*: 1, oxygen data against temperatures obtained from cherry tree blooms; 2, change of the first freezing date of Lake Suwa; 3, change of number of snow days a year, calibrated by comparison with modern changes and modern temperatures. *c*, D/H ratio and ^{18}O/^{16}O ratio against time, the least squares fits against time, and the least squares fit to the dependence of δD on δ^{18}O with a slope of 8.2.

Further, we have measured an old Japanese cedar, *Cryptomeria japonica* or Yaku-Sugi (the ^{14}C in the rings of which were measured by Kigoshi[17]), for both hydrogen and oxygen isotope ratios. Kigoshi counted the rings of this tree and verified the count by making 50 radiocarbon datings on them. Ten dates were made on the oldest part of the tree, each to an accuracy of ±100 yr, for the years counted as AD 137–537. The accuracy of his count in the oldest part is thus substantiated to an accuracy of $\pm100/\sqrt{10}$ or ±30 yr, by the radiocarbon evidence. In Fig. 5 we have compared the modern part of the cedar with temperatures[18] measured at Miyazaki, a nearby weather station. Although Miyazaki is at sea level, our *Cryptomeria* grew at a higher altitude, about 5,000 feet. In spite of climatic differences which might be expected to arise from difference in altitude, there seems to be significant agreement, allowing us to calibrate the isotope changes in terms of temperature differences.

Measurements of the isotopic composition of atmospheric moisture are compared, in Fig. 6 *a* and *b*, with temperatures deduced from surrogate evidence obtained from old diaries for Japan[19] and for China[20]. Figure 6*a* raises several questions. Are the tree ring ages correct in this tree? Is the surrogate evidence giving rise to the Yamamoto and Chu comparison temperatures and to the Norwegian snow line data either pertinent or correct? It may be possible to find other evidence for the temperature changes indicated by the isotope variations in the cedar and thus indirectly check the method further.

The fact that

$$\delta D = 8.2\delta^{18}O + \text{constant}$$

for the cedar, showing a slope consistent with that for worldwide rainwater within experimental error[12,21], indicates that the tree is storing old rainwater. Furthermore, it supports the hypothesis that the CO$_2$ and H$_2$O are in isotopic equilibrium in the atmosphere.

The absolute values of the deuterium ratios, about −70 SMOW, are about the same as in rainwater. The absolute values of the oxygen ratios, around +20 SMOW, are halfway between that for rain (about −7 SMOW), and that for atmospheric CO$_2$ (about +40 SMOW). This is to be expected for water vapour in equilibrium with atmospheric carbon dioxide and keeping in mind that carbon dioxide absorbed by the leaves exchanges its oxygen with sap.

We have made Fourier transforms of the data in Fig. 5*c* to deduce the power spectrum of periodicities. The results are shown in Fig. 7 *a* and *b*, and in Table 1. The same periods are found in both D/H and in $^{18}O/^{16}O$ data, within experimental error, as obtained from the widths of the peaks in the power spectra.

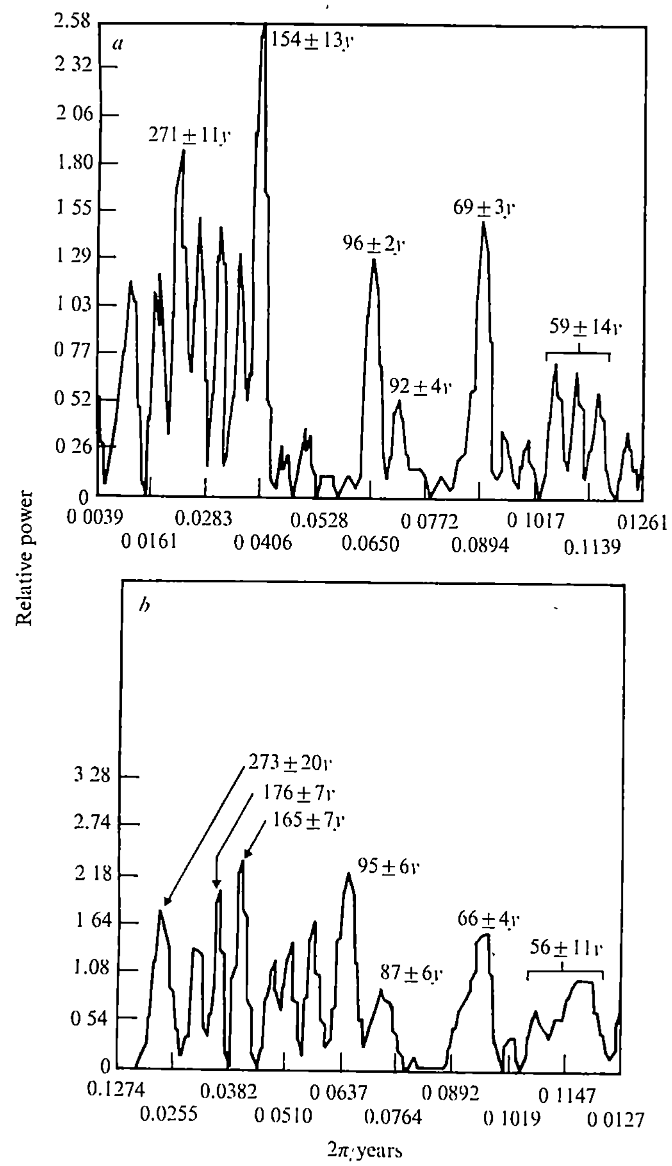


Fig. 7 Power spectra obtained from Fourier transforms of the data in Fig. 6*c*. The indicated periods are not sensitive to various ways of cutting off the ends of the data. *a*, Fourier transform of $\delta^{18}O$, average slope subtracted; *b*, Fourier transform of δD, average slope subtracted.

Table 1 Periods and their respective powers found in Fourier transforms of oxygen and hydrogen stable isotope data in Japanese Cedar AD 140–1950

$\delta^{18}O$ Period (yr)	$\delta^{18}O$ Relative power	δD Period (yr)	δD Relative power
59±14	0.67	56±11	1.00
69±3	1.50	66±4	1.64
92±2	0.52	87±3	1 00
96±2	1.29	95±2	2.18
154±6	2.58	153±7	2.18
174±7	1.29	174±8	2.00
202±7	1.45	205±9	1.70
271±11	1.80	273±20	1.64

Our samples represented approximately 5 yr each, so we cannot expect to find evidence for periods of less than about 40 yr in these data. As the sets of data span only about 1,800 yr, we cannot expect to find evidence for periods of more than about 250 yr. Both data sets show a long term decline of about 1.6 °C in average temperature over the past 1,800 yr. This is significant, especially considering that there was a decline of about 10 °C in the last ice age. In making the Fourier transforms we have used the deviations of the isotope ratios from the long term slopes—that is, we have corrected for the slope. One may ask whether the periods of about 58 yr and about 68 yr are really different from each other, and likewise for the periods of about 90 yr and 95 yr. The peaks in Fig. 7 suggest that they are. We need to measure more trees to find out. We varied the methods of cutting off the data at the end points to confirm that the period of 272 yr is real. Also we fed artificial periodicities of 50 and 55 yr and of 90 and 95 yr into the Fourier transform and found them separable.

Future research

It seems wise to measure more modern ring sequences and compare them with local mercury thermometer records in order to establish the tree thermometer phenomenon beyond question. And, of course, there are experiments to be done growing woody material in conditions of controlled temperature and isotopic ratios in atmosphere and water. We plan also to study old tree ring sequences. We have such sequences in our laboratory, including sequoias, firs, cedars, and pines provided by B. Becker, Dieter Eckstein and other experts on tree ring counting.

When we have measured isotope ratios in each ring of a lengthy sequence (as opposed to making measurements of five rings at a time) we shall explore the Fourier transforms of the data for evidence of the sunspot cycle of about 21 yr and other periodicities of less than 50 yr and for sum and difference frequencies. When we have determined whether there are shorter periodicities, and substantiated the longer periodicities listed in Table 1, we plan to put them in the phase relationships indicated by the trees and predict the trends of climate to be expected for the next twenty years or so.

The exciting possibility of studying trees felled by the advancing ice sheet at the Two Creeks site in Wisconsin 12,000 yr ago is an obvious application of this technology. It seems likely that if the method is sound we can establish the history of climate back many millennia, as far back, in fact, as there are tree remnants to be found.

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Stratospheric ozone as viewed from the Chappuis band

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Stratospheric total ozone values have been obtained for the period 1912–50 from analysis of Smithsonian data. Naturally caused variations of 25% or more are common over time scales ranging from months to decades. We suggest that the ozone layer acts as a shutter on the incoming solar energy, providing one of the long sought trigger mechanisms between solar activity and climatic change. The present state of knowledge of ozone climatology is not sufficient to support popular speculations about man's effects on the ozone layer.

MUCH speculation has been published about the naturally occurring ozone in the Earth's stratosphere. The controversy has centred around theoretical attempts to demonstrate that certain of man's activities such as the flight of SSTs or the production and release of fluorocarbons will reduce the mean amount of naturally occurring ozone and lead to unwanted biological effects[1]. These theoretical speculations generally predict a total depletion of nearly 7%. Because of its absorption bands in the ultraviolet, visible and infrared parts of the spectrum, ozone is also an important climatological parameter. Thus it is particularly interesting to examine the history of natural variations in ozone amounts over a time span of several decades.

We are trying to determine historical baselines for atmospheric constituents using data obtained by the Smithsonian Institution's Astrophysical Observatory (APO) in their solar constant observing programme from 1902 to 1961. As a normal part of their observing procedure, they obtained spectrobolometric tracings of the direct solar radiation reaching the ground over the spectral range 0.35–2.2 μm. We have previously analysed the published portion of these data for changes in atmospheric transmission caused by suspended aerosols[2]. We have now extended our analysis of these data to determine values for total stratospheric ozone above high altitude stations in southern California from 1912–1950 and northern Chile from 1918–1948.

In the past, virtually all long term monitoring programmes for total ozone have used Dobson spectrophotometers or other instruments such as filter photometers. These instruments operate in the ultraviolet part of the spectrum to observe the strong ozone absorption in the Huggins bands (0.30–0.35 μm). The APO transmission measurements included the Chappuis band (0.5–0.7 μm) and therefore offer both an extension of the baseline in time and place, and a valuable check on the Dobson ozone amounts determined with this different band. The measured transmission in the Chappuis band has been reduced by ozone, Rayleigh scattering, and aerosol scattering. We used the transmission data outside the ozone band to calculate the Rayleigh and aerosol components. Absorption coefficients from Vigroux[3] were then used to calculate the total ozone.

The Chappuis band has generally not been used for ozone because it is a very weak feature. The APO data are, however, of sufficient quality that we were able to model the Rayleigh and aerosol contributions so that the resulting daily probable error in ozone amounts was only 7%. An advantage of the Chappuis band is that its absorption coefficient is unaffected by temperature[3]—a property not shared by the ultraviolet bands.

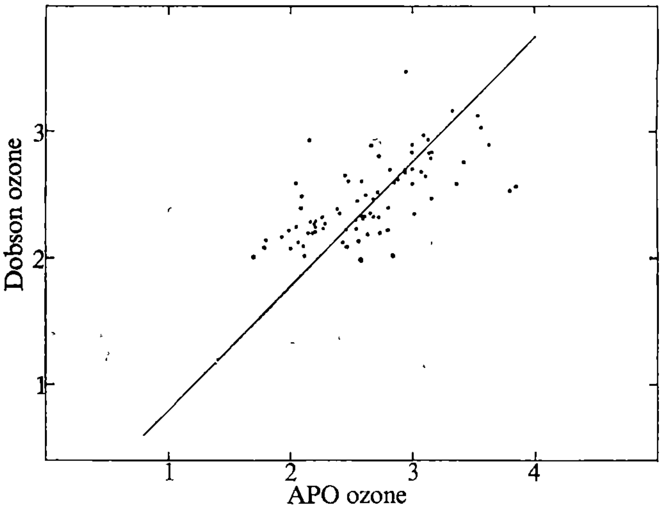

Fig. 1 Correlation between ozone amounts determined from 78 d of observation at Table Mountain, California with both a Dobson spectrometer and an APO spectrobolometer. Ozone concentrations here and in subsequent Figs are measured in mmHg (NTP).

A comparison between Dobson values and our values is possible from 78 d of nearly simultaneous APO and Dobson observations made at Table Mountain, California from August, 1928 to May, 1929 (Fig. 1). A simple linear regression analysis yields a correlation coefficient of 0.7 and a slope of 0.98 between the two data sets. The lack of perfect agreement is attributed to several causes. First, the APO observations were carried out a few hours earlier on each day than the Dobson work. Second, the ozone absorption coefficients are not precisely known; inaccuracies of up to 10% may still exist[4]. Third, the calculated error for values derived from the daily APO measurements is 0.23 mm s.d. in the APO–Dobson comparison. It is for this last reason that we present here monthly mean values of ozone, where the calculated error is reduced typically to ~0.10 mm.

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Figures 2 and 3 show monthly mean and 12-month running mean total ozone amounts for southern California and northern Chile, expressed in terms of the equivalent height of a vertical column of pure ozone at NTP. Each point before 1930 represents the mean of $\sim$6 d. Observations were made at Mt Wilson only during the summer months. Only $\sim$50 values per site were published for the period 1940–1950; thus each point in that period represents typically 1 or 2 d compared to typically 6 d for the earlier period.

It is apparent from Figs 2 and 3 that at both sites total ozone amounts commonly show variations of as much as 20–30% on time scales ranging from months to decades. Comparison with other work[5] shows that such variations are not at all unusual in Dobson spectrophotometry. We also present 12-month running means (dashed line) for comparison with the highly smoothed data usually found in the literature. Even here large variations are found that are comparable to those recently reported by other workers. For instance, Angell and Korshover[6] found changes of up to 8% over 3 yr, and Komhyr *et al.*[7] found changes from 2–10% per decade for the period 1960–70. We find that such changes occurred throughout the forty-year APO record analysed here.

Because the large natural variations in the ozone layer are not uncommon, consideration of the amount of incident solar energy absorbed by the Chappuis band suggests a new perspective on the long sought link or 'trigger mechanism' between solar activity and the Earth's weather and climate[8]. Stratospheric ozone is created photochemically by solar radiation in the wavelength region from $\sim$1,800 to 2,100 Å (ref. 9). Although the solar energy output shows substantial variations in this region and at shorter wavelengths[10], this total energy change is such a small fraction of the average solar output that it is generally considered to fall far short of meteorological significance. Also, photons in this wavelength region are absorbed in the stratosphere, requiring that any tropospheric effects should be indirect[8]. Since the ultraviolet Hartley and Huggins bands are saturated and Rayleigh and aerosol scattering are very large in the ultraviolet, the net atmospheric transmission of radiation in the near ultraviolet is not strongly affected by variations in stratospheric ozone.

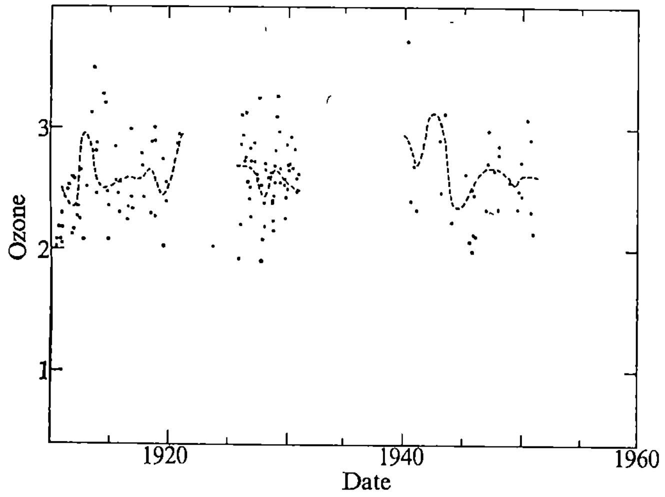

Fig. 2 Monthly mean values of total ozone for the southern California sites Mt Wilson (1912–20) and Table Mountain (1925–30 and 1940–50). The dashed line represents twelve month running means. Typical probable error is 0.10 mm.

On the other hand, because of its location near the peak of the solar energy spectrum, the Chappuis band absorbs on average $\sim$2% of the total solar flux at midlatitudes[11]. Thus, for example, if solar variations and stratospheric transport combine to produce an increase in stratospheric ozone of 25% over a given mid-latitude station, the total solar energy reaching the troposphere above that station would be decreased by $\sim$0.5%, an amount of importance to both weather and climate. Thus ozone can serve as a stratospheric shutter operated by ultraviolet rays but modulating the energy flux in the visible region. Because of the longer slant path lengths and larger amounts of ozone, the effect is much larger in the polar regions. Ozone climatology is further complicated by the absorption band at $9.6\ \mu m$ which makes an important contribution to the greenhouse effect[12,13].

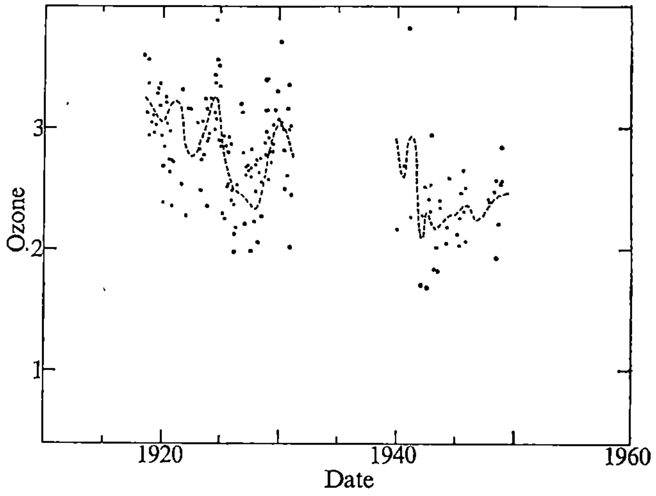

Fig. 3 Monthly mean total ozone for northern Chile sites Calama (1918–20) and adjacent Mt Montezuma (1920–30 and 1940–48). The dashed line represents twelve month running means. Typical probable error is 0.10 mm.

The possibility that man might inadvertently modify the ozone level is a matter of much concern. The presence of large variations in ozone amounts makes it clear that we must really think in terms of a natural range in ozone amounts, rather than a single level. Considering the magnitude of the spatial and temporal variations involved, there exists only a relative handful of measurements of past ozone levels, and it is not now possible to say what might constitute an optimum ozone level at a particular time and place, or if such a level can even be defined. Finally, we note that because of the presence of natural biological processes, it is questionable whether man can permanently modify the ozone layer[14,15].

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Evidence for role of m^{7}G$^{5'}$-phosphate group in recognition of eukaryotic mRNA by initiation factor IF-M$_3$

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7-methylguanosine 5′-monophosphate inhibits protein synthesis in a fractionated, messenger-dependent, reticulocyte cell-free system. This compound also inhibits binding of histone mRNA to reticulocyte ribosomes as well as interaction of VSV mRNA and histone mRNA but not EMC virus RNA with purified initiation factor IF-M$_3$. These studies provide evidence that the role of 7-methylguanosine in the mechanism for initiation of eukaryotic mRNA translation may be related to specific recognition of mRNA by initiation factor IF-M$_3$.

WITHIN the past two years a number of investigators have identified a unique 5′-triphosphate linkage of 7-methylguanosine (m^{7}G) to the 5′-terminal nucleotide of various eukaryotic mRNAs[1-12]. It has also been reported[7] that chemical removal of m^{7}G from rabbit globin mRNA and the absence of either the 7-methyl group or m^{7}G in reovirus mRNA[13] results in reduced ability of these RNAs to serve as templates for protein synthesis. Hickey *et al.*[14] have found in a wheat germ cell-free system that 7-methylguanosine-5′-monophosphate (m^{7}G$^{5'}$p) inhibits translation of several eukaryotic mRNAs containing the 5′terminal m^{7}Gppp... group. Translation of satellite tobacco necrosis virus RNA, a plant virus RNA with a free 5′-terminal phosphate (see ref. 14), was not, however, inhibited by m^{7}G$^{5'}$p. From these and other studies it was concluded that m^{7}G$^{5'}$p acted as an inhibitor of mRNA addition to the initiation complex[14].

In the present study we have tested the effects of m^{7}G$^{5'}$p on protein synthesis in the rabbit reticulocyte cell-free system. In preliminary experiments m^{7}G$^{5'}$p was added to unfractionated reticulocyte lysate. In these conditions, there was only a slight inhibition of protein synthesis at high concentrations of this nucleotide (1.25 mM m^{7}G$^{5'}$p produced only a 15% inhibition). This is in contrast to our previous results with unfractionated wheat germ extract in which 0.125 mM m^{7}G$^{5'}$p inhibited mRNA translation by more than 90% (ref. 14). A major difference between these systems is that wheat germ extract is dependent on exogenous mRNA for protein synthesis, whereas reticulocyte lysate contains large quantities of endogenous mRNA already associated with polysomes. We have, therefore, used a fractionated, reticulocyte mRNA and initiation factor dependent, cell-free system to study the mechanism of inhibition of protein synthesis by m^{7}G$^{5'}$p. With this system under the direction of homologous reticulocyte mRNA, m^{7}G$^{5'}$p produces a marked inhibition of protein synthesis. In addition, m^{7}G$^{5'}$p specifically inhibits mRNA from entering into a stable 40S or 80S "initiation complex" with reticulocyte ribsomal subunits. Finally, we show that m^{7}G$^{5'}$p inhibits the interaction of certain mRNAs with purified reticulocyte initiation factor IF-M$_3$.

Inhibition of protein synthesis

A cell-free protein synthesising system, dependent on exogenous mRNA and initiation factors, was prepared from rabbit reticulocytes by treating polysomes with low concentrations of pancreatic RNase, followed by washing the ribosomes with 0.5 M KCl and sedimenting them through a 1.0 M sucrose cushion[15]. Other reticulocyte components were prepared as reported previously[16]. This system has been characterised previously[15] and demonstrates all the steps necessary for synthesis of authentic polypeptides with relatively high efficiency and linear incorporation of amino acids into protein for up to 1 h (ref. 16). With saturating levels of exogenous reticulotype poly(A)$^+$ mRNA and initiation factors, m^{7}G$^{5'}$p produced a marked reduction in ^{14}C-leucine incorporation (Fig. 1). Inhibition of protein synthesis by m^{7}G$^{5'}$p was not reversed by threefold excess initiation factors added separately or as a crude mixed factor preparation. Little inhibition was observed with either 7-methylguanosine or 7-methylguanosine-2(3)′-monophosphate (Fig. 1). Since globin mRNA has been found to contain 5′-terminal m^{7}Gppp... (refs 7 and 13), these results suggest that inhibition of exogenous mRNA translation by m^{7}G$^{5'}$p is related to a structural homology between m^{7}G$^{5'}$p and the 5′-terminus of this eukaryotic mRNA.

Inhibition of initiation complex formation

To study the mechanism of inhibition of protein synthesis by m^{7}G$^{5'}$p, purified initiation factors and radioactive mRNAs have been used. In this way the interaction of mRNA with ribosomal subunits and initiation factors could be followed directly. For this purpose we have used a radioactive cellular mRNA, histone mRNA, which was shown previously to be translated faithfully by the reticulocyte cell-free system[18-19]. ^{3}H-histone mRNA was incubated with unfractionated rabbit reticulocyte lysate in protein synthesis conditions but in the presence of 0.2 mM sparsomycin to inhibit movement of labelled mRNA into large polyribosome structures (L.A.W., M. C. Williams, and C. B., unpublished). After a brief incubation at 30 °C, ribosomes and ribosomal subunits were separated by sucrose gradient centrifugation. Association of mRNA with ribosomes was determined by the appearance of label in sucrose gradient fractions compared with the ribosome A_{260} profile. Binding of ^{3}H-histone mRNA to reticulocyte ribosomes occurred rapidly in these conditions (Fig. 2*a*) and 68% of the added label sedimented in the 80S region after 2 min of incubation. Little ^{3}H-material appeared in the 40S subunit region,

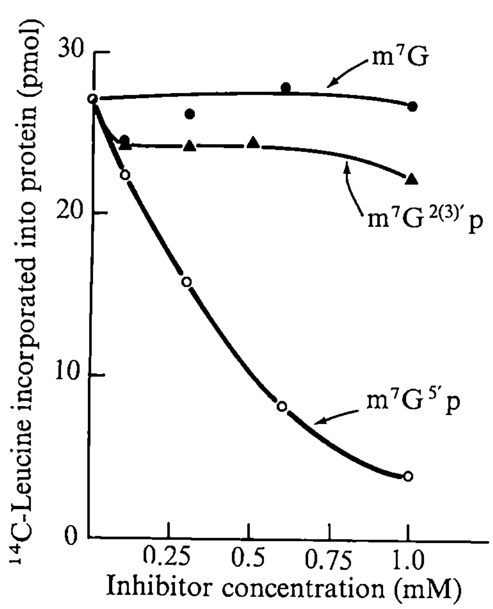

Fig. 1 Inhibition of protein synthesis in reticulocyte messenger-dependent cell-free system by 7-methylguanosine 5′-monophosphate. The preparation of rabbit reticulocyte lysate, 0.5 M KCl-washed ribosomes, 105,000*g* supernatant protein and crude initiation factors was as previously reported[16]. Ribosomes were rendered dependent on exogenous mRNA by incubation with very low levels of pancreatic RNase as previously described[15]. Messenger RNA (RNA which contains a 3′-terminal poly(A) sequence) was prepared from reticulocyte polysomes by standard procedures, including multiple extractions with phenol–chloroform–isoamyl alcohol and adsorption of poly(A)$^+$ material to oligo(dT)–cellulose according to the method of Aviv and Leder[17]. For the complete system, incubations in a total volume of 50 µl were performed at 30 °C for 30 min and contained 15 mM Tris-HCl, *p*H 7.4 ,80 mM KCl, 3 mM $MgCl_2$, 1 mM ATP, 0.2 mM GTP, 3 mM phosphoenolpyruvate, 0.2 IU of pyruvate kinase (rabbit muscle), 1 mM dithiothreitol, 80 µM L-amino acids minus leucine, L-^{14}C-leucine (specific activity 80 mCi mmol^{-1}), 0.1 A_{260} unit of 0.5 M KCl-RNase treated reticulocyte ribosomes, 0.06 A_{260} units deacylated rabbit liver tRNA, 300 µg of 105,000*g* supernatant protein, 150 µg of crude reticulocyte initiation factors, and 0.2 A_{260} units of partially purified reticulocyte mRNA. Where indicated, 7-methylguanosine (m^7G) (●), 7-methylguanosine 2(3)′-monophosphate ($m^7G^{2(3)'}p$) (○), or 7-methylguanosine 5′-monophosphate ($m^7G^{9'}p$) (▲) were added to the complete system. Reactions were stopped by the addition of ice-cold 10% trichloroacetic acid and protein synthesis determined by incorporation of ^{14}C-leucine into hot trichloroacetic acid-insoluble material[16].

and unbound ^{3}H-histone mRNA remained near the top of the gradient. In a control experiment ^{3}H-histone mRNA was added to an incubation mixture kept at 0 °C. This did not result in binding of mRNA to ribosomes or ribosomal subunits (data not shown). Addition of 0.25 mM $m^7G^{5'}p$ to the incubation mixture (Fig. 2*b*) resulted in a 36% reduction of ^{3}H-histone mRNA binding to ribosomes and 1 mM $m^7G^{5'}p$ produced a 75% reduction in ^{3}H-histone mRNA binding (Fig. 2*c*). $m^7G^{5'}p$ did not cause a shift in ^{3}H-histone mRNA binding from the 80S to the 40S region (Fig. 2*b*, *c*).

Various investigators have reported that Met-tRNA$_f$ forms a ternary complex with GTP and a specific initiation factor IF-MP[20] (also referred to as IF-I[21,22], IF-E2[23], and IF-L3[24]). Formation of this complex is thought to lead to Met-tRNA$_f$ association with the 40S subunit independent of mRNA, and it has been reported that Met-tRNA$_f$–40S subunit interaction is required for incorporation of mRNA into the "initiation complex"[23-25]. To measure formation of the Met-tRNA$_f$–40S complex, purified rabbit reticulocyte Met-tRNA$_f$ charged with ^{3}H-methionine (3 Ci mmol^{-1}) and the sucrose gradient assay described by Darnborough *et al.*[25] were used. As shown in Fig. 3, binding of ^{3}H-Met-tRNA$_f$ to the 40S ribosomal subunit is not inhibited by $m^7G^{5'}p$. From these studies (Fig. 2 and Fig. 3) we conclude that $m^7G^{5'}p$ inhibits protein synthesis by preventing mRNA from forming a stable complex with ribosomes or ribosomal subunits, but this does not occur by inhibition of Met-tRNA$_f$–40S subunit complex formation.

Effect of $m^7G^{5'}p$ on IF-M3-mRNA interaction

To determine whether $m^7G^{5'}p$ might inhibit protein synthesis by competing with mRNA for a common binding site on one of the initiation factors, a direct assay of mRNA-protein interaction was used. Hellerman and Shafritz[26] reported that reticulocyte initiation factor IF-MP forms a complex with ^{3}H-labelled rabbit globin mRNA. This complex was demonstrated by its retention on a nitrocellulose filter, in conditions in which free mRNA passed through the filter. A similar complex between IF-MP and bacteriophage R17-RNA has been reported by Kaempfer[27]. More recently, using labelled vesicular stomatitis virus (VSV)-mRNA or duck globin mRNA of much higher specific activity, we have found that reticulocyte initiation factors IF-M$_3$ and IF-M$_{2A}$ also form complexes with mRNA. Other purified reticulocyte initiation factors form such complexes to a much lesser extent (to be reported in detail elsewhere).

For experiments of this type, as well as these reported below, highly purified initiation factors are required. Details for the purification of these factors have been published[20,28-30] and materials used in the present study were prepared as follows: IF-MP was purified up to and including step 5 in ref. 20, IF-M$_{2A}$ up to and including step 6 in ref. 28, IF-M$_{2B}$ up to and including step 5 in ref. 29, and IF-M$_3$ up to and including step 5 in ref. 30, plus an additional purification by ion exchange chromatography with phosphocellulose in HEPES buffer at *p*H 6.8. This IF-M$_3$ preparation contains predominantly one polypeptide component (~80–90%) of molecular weight ~80,000 on sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis and seems to correspond to factor IF-E6 of Staehelin *et al.*[23].

In the present study, we have examined mRNA–protein complex formation with ^{3}H-histone mRNA, ^{3}H-VSV mRNA

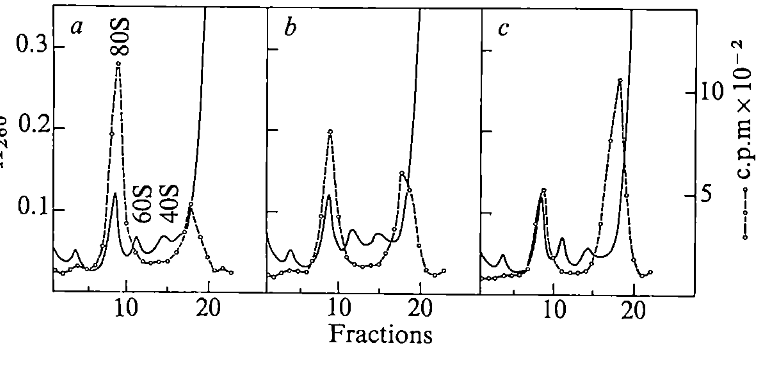

Fig. 2 Inhibition of histone mRNA binding to reticulocyte initiation complexes by 7-methylguanosine-5′-monophosphate. For each 25 µl incubation of unfractionated reticulocyte lysate, 5,000 c.p.m. of ^{3}H-histone mRNA (specific activity > 200,000 c.p.m. µg) were added together with 0.1 mM sparsomycin and $m^7G^{9'}p$, where indicated. Sparsomycin was added specifically to bind mRNA to 80S initiation complexes, since this drug prevents elongation, but does not inhibit initiation[25]. At the end of the incubation 0.4 ml of ice-cold 10 mM NaCl, 10 mM Tris *p*H 7.4 and 1.5 mM $MgCl_2$ were added and the samples fractionated by 16 h centrifugation at 22,000 r.p.m. on 15–30% sucrose gradients prepared in the same buffer. Fractions of 0.8 ml were collected and counted directly by addition of 15 ml of scintillation fluid containing 1 volume Triton X-100, 2 volumes of 0.8% butyl-PED (CIBA) and 0.3 volume water. *a*, Control, no $m^7G^{9'}p$ added. *b*, 0 25 mM $m^7G^{9'}p$ added. *c*, 1.0 mM $m^7G^{5'}p$ added.

and ^{3}H-encephalomyocarditis (EMC) virus RNA. Histone mRNA and VSV mRNA contain "blocked", methylated 5′-termini (ref. 31 and B. Moss, personal communication), whereas EMC virus RNA contains an unblocked 5′-terminal structure[32]. In addition, histone mRNA does not contain 3′-terminal poly(A)[33]. As shown in Table 1, complexes can be demonstrated between all three ^{3}H-mRNAs and initiation factors IF-MP, IF-M$_3$ and IF-M$_{2A}$ but not with IF-M$_{2B}$. Inhibition of mRNA-protein complex formation by m^{7}G$^{5'}$p is found exclusively with IF-M$_3$. Assuming a molecular weight of 2.7×10^6 daltons for EMC virus RNA and an average molecular weight of 0.5×10^6 for 12–18S VSV mRNA and roughly equivalent percentages of uridine in these molecules, complex formation between IF-M$_3$ and EMC virus RNA on a molar basis is approximarely one-fourth that obtained with VSV mRNA. In addition, complex formation between ^{3}H-EMC virus RNA and IF-M$_3$ is not inhibited by m^{7}G$^{5'}$p (Table 1). Since all three mRNAs form complexes with initiation factors IF-MP, IF-M$_3$ and IF-M$_{2A}$, a 3′-poly(A) sequence does not seem to be required for the observed phenomena.

It is important to note that highly purified IF-M$_3$ is required to obtain the observed degree of inhibition with m^{7}G$^{5'}$p; only 30–40% inhibition is found with less purified material. Inhibition of IF-M$_3$–mRNA complex formation also seems to be specific for m^{7}G$^{5'}$p, as other guanosine nucleotides tested do not produce this effect (Table 2). Together with the experiments reported in Figs 2 and 3, these results indicate that inhibition of protein synthesis by m^{7}G$^{5'}$p correlates with the ability of this compound to prevent interaction of mRNA with IF-M$_3$. Presumably, IF-M$_3$–mRNA interaction is required for mRNA association with the 40S ribosomal subunit, but as yet we have not developed an assay system to test this directly.

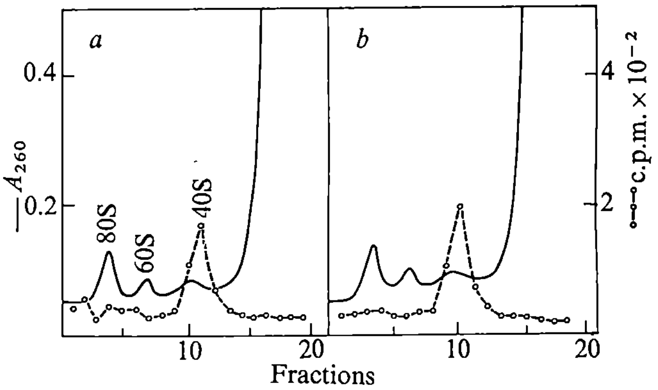

Fig. 3 Binding of ^{3}H-Met-tRNA$_f$ to the small ribosomal subunit in unfractionated rabbit reticulocyte extract in the absence (*a*) or presence (*b*) of m^{7}G$^{5'}$p. The binding reactions were carried out according to Darnbrough *et al.*[25] in 0.1 ml of the complete reticulocyte lysate system. To each incubation was added 0.1 mM sparsomycin and 1 mM m^{7}G$^{5'}$p was added to *b*. After 2 min at 30 °C, 3 pmol of ^{3}H-Met-tRNA$_f$ (1,500 c.p.m. pmole^{-1}) from rabbit reticulocytes were added for 2 min. The incubations were terminated by the addition of 0.4 ml of ice-cold 10 mM NaCl, 10 mM Tris *p*H 7.4 and 1.5 mM MgCl$_2$. The samples were fractionated by centrifugation on 15–30% sucrose gradients in the same buffer for 16 h at 22,000 r.p.m. in the Beckman SW27 rotor. 0.8-ml fractions were collected and counted after addition of 0.25 mg carrier yeast tRNA and precipitation with 1.5 ml of 2% acetyltrimethylammonium bromide. The precipitates were collected on Millipore filters and counted as in Fig. 2.

Relationship of m^{7}Gppp . . . to the eukaryotic initiation process

In spite of many recent advances in our understanding of the mechanism of initiation of eukaryotic protein synthesis, little progress has been made in elucidating how mRNA is recognised by the ribosome or how specific factors might participate in selecting specific mRNAs or particular nucleotide sequences of mRNAs. To approach this problem, we have begun to study the properties of proteins associated with mRNA and the relationship of these proteins to reticulocyte initiation factors. We reported that IF-MP activity is present on messenger

Table 1 Inhibition by m^{7}G$^{5'}$p of binding of ^{3}H-uridine-labelled mRNAs to reticulocyte initiation factors

Factor	^{3}H-VSV mRNA bound (c.p.m.)		^{3}H-histone mRNA bound (c.p.m.)		^{3}H-EMC virus RNA bound (c.p.m.)	
	Control	+ m^{7}G$^{5'}$p	Control	+ m^{7}G$^{5'}$p	Control	+ m^{7}G$^{5'}$p
IF-MP	2,710	2,580	872	919	16,588	13,917
IF-M$_3$	1,930	643	796	69	18,261	22,059
IF-M$_{2A}$	2,618	2,411	452	360	40,429	36,614
IF-M$_{2B}$	126	—	23	—	197	284

Binding of ^{3}H-VSV mRNA, ^{3}H-histone mRNA and ^{3}H-EMC virus RNA to purified reticulocyte initiation factors was determined by the nitrocellulose filter assay reported previously[26]. ^{3}H-VSV mRNA was prepared from virus-infected HeLa cells incubated with ^{3}H-uridine by the method of Grubman *et al.*[35]. This RNA contains poly(A), sediments between 12S and 18S, and has been shown to have m^{7}G$^{5'}$ppp at the 5′-terminus[31]. ^{3}H-histone mRNA was prepared from HeLa cells synchronised in S phase and incubated with ^{3}H-uridine. The histone mRNA was isolated by phenol extraction and repeated sucrose gradient centrifugation (L. A. W , M. C. Williams, and C.B., unpublished). Histone mRNA, labelled with ^{3}H-methylmethionine and isolated as described above, has been shown to have a blocked 5′-terminus (B. Moss, personal communication). The ^{3}H-methylhistone mRNA was enzymatically digested and analysed by chromatography on DEAE–cellulose in 7 M urea at *p*H 7.6. About 80% of the c.p.m. applied were eluted with a charge of −5 or −6, which is the position expected for blocked 5′-termini[36]. Encephalomycarditis (EMC) virus RNA was prepared from HeLa cells. The cells were concentrated to 4×10^7 ml^{-1} in serum-free MEM medium containing 14 mM HEPES buffer *p*H 7.4 and 2 mM glutamine. EMC virus was added at a multiplicity of 10 PFUs cell^{-1} for 15 min at 23 °C. The culture was diluted tenfold with warm medium containing 7% foetal calf serum and 5.5 μg ml^{-1} actinomycin D. After 30 min at 37 °C ^{3}H-uridine (26.2 Ci mmol^{-1}) was added to a final concentration of 50 μCi ml^{-1}. After 14 h, the culture was frozen and thawed three times and centrifuged 10 min at 10,000*g*. The supernatant was neutralised with 1 M Tris and made 6% in polyethylene glycol The precipitate which formed after stirring for 16 h at 4 °C was collected by 15 min centrifugation at 15,000*g* and resuspended in 2 ml of 0.1 M NaCl, 10 mM Tris *p*H 7.5 and 10 mM mercaptoethanol. This suspension was incubated for 20 min at 37 °C with 50 μg ml^{-1} of trypsin and SDS added afterwards to 0 5%. Virus was purified by 2-h centrifugation at 150,000*g* through a 2-ml layer of 15% sucrose in SDS–buffer[37]. The pellet was resuspended in SDS–buffer and the RNA extracted with phenol–chloroform[37]. RNA was recovered by ethanol precipitation and was purified by 15-h centrifugation at 25,000 r.p.m. on a 15–30% sucrose gradient. The 35S viral RNA was precipitated with ethanol and reprecipitated twice. The EMC virus RNA has a specific activity of 380,000 c.p.m. μg^{-1}. Incubations in a total volume of 50 μl were performed at 23 °C for 5 min and contained 20 mM Tris-HCl, *p*H 7.4, 100 mM KCl, 1 mM dithiothreitol and 0.53 μg IF-MP, 3.5 μg IF-M$_3$, 1.1 μg IF-M$_{2A}$ or 0.99 μg IF-M$_{2B}$. In each case, 4,000–5,000 c.p.m. ^{3}H-VSV mRNA (specific activity 450,000 c.p.m. per A_{260} unit), 3,000 c.p m. of ^{3}H-histone mRNA (specific activity > 200,000 c.p.m. μg^{-1}) or 60,000 c p.m. ^{3}H-RMC virus RNA (specific activity 380,000 c.p.m. μg^{-1}) was included as substrate and reactions were performed either in the absence (control) or presence of 1.0 mM m^{7}G$^{5'}$p. Reactions were stopped by the addition of 3 ml of ice-cold buffer solution (20 mM Tris-HCl, *p*H 7.4, 100 mM KCl). RNA–protein complexes were collected on HAWP 0.45 μm pore size, 25 mm diameter, nitrocellulose filters (Millipore) and rinsed three times with 3 ml of the same buffer. The filters were dissolved in 10 ml Bray's solution[38] and counted by liquid scintillation spectroscopy at 23% efficiency for ^{3}H. Blanks of 175 c.p.m., 84 c.p.m. or 671 c.p.m., representing isotope retension on the nitrocellulose filter in the absence of added protein factors, were subtracted from the appropriate values.

* For experiments with ^{3}H-EMC virus RNA, the amount of the protein factors was reduced to one-third that used for the other mRNAs.

ribonucleoprotein particles (mRPNs) obtained from reticulocyte polysomes and that purified IF-MP can interact with globin mRNA to form an mRNA–protein complex[26]. In the present studies, we have found that in addition to IF-MP, purified initiation factors IF-M$_3$ and IF-M$_{2A}$ can also form complexes with mRNA. Of the various factors tested, however, only the interaction of IF-M$_3$ with mRNA is inhibited by m^{7}G$^{5'}$p. This inhibition requires both the 7-methyl and the 5′-phosphates moieties, and the requirements for inhibition of IF-M$_3$–mRNA complex formation are the same as those for inhibition of overall protein synthesis.

The amount of m^{7}G$^{5'}$p required for inhibition of either protein synthesis or IF-M$_3$–mRNA interaction is far in excess of the amount of mRNA present in the system (greater than 1,000/1). Therefore, the interaction of m^{7}G$^{5'}$p with IF-M$_3$ seems to represent a low affinity binding and the 5′-terminal region of natural mRNAs recognised by IF-M$_3$ may contain additional nucleotides. Since we are limited in the amount of specific initiation factors we can add to the cell-free system, the high ratio of m^{7}G$^{5'}$p to IF-M$_3$ may explain our inability to obtain reversal by excess IF-M$_3$. It should also be noted that endogenous protein synthesis with unfractionated reticulocyte lysate is only slightly inhibited by m^{7}G$^{5'}$p. In the reticulocyte lysate system, however, m^{7}G$^{5'}$p does inhibit association of exogenous histone mRNAs with ribosomes (Fig. 2), as well as translation of exogenous histone mRNA (our unpublished observations). At the moment, we have no explanation for the low inhibition of endogenous protein synthesis by m^{7}G$^{5'}$p in reticulocyte lysate. One possible interpretation of these findings is that endogenous mRNA in reticylocyte lysate is already associated with specific proteins (such as IF-M$_3$) and that translation of performed mRNA protein complexes cannot be inhibited by m^{7}G$^{5'}$p, whereas translation of added deproteinised mRNA can be. We hope to test this hypothesis in future experiments by comparing the effect of m^{7}G$^{5'}$p on translation of exogenous reticulocyte mRNA compared with reticulocyte mRNP in the fractionated messenger dependent cell-free system.

Table 2 Specificity for inhibition of ^{3}H-VSV mRNA-IF-M$_3$ complex formation by guanosine nucleotides

Inhibitor added	c.p.m. ^{3}H-VSV mRNA bound	% Inhibition
None	2,260	—
m^{7}G	1,900	16
m^{7}G$^{2(3)'}$p	1,965	13
m^{7}G$^{5'}$p	800	65
G$^{5'}$p	2,021	10

For incubation conditions and assay procedure see Table 1. Where indicated, incubations were performed in the presence of 1.0 mM m^{7}G, m^{7}G$^{2(3)'}$p, or m^{7}G$^{5'}$p, G$^{5'}$p added before 3H-VSV mRNA.

From the present studies, it seems that association of mRNAs containing m^{7}Gppp . . . with initiation factor IF-M$_3$ may be essential for binding of certain mRNAs to the ribosome. In addition, inhibition of IF-M$_3$–mRNA interaction by m^{7}G$^{5'}$p may be the result of competition between this nucleotide and mRNA for a specific recognition site on IF-M$_3$. Since m^{7}G$^{5'}$ppp . . . has been identified as the 5′-terminal group in various eukaryotic mRNAs, a normal function of IF-M$_3$ might be to form a complex with certain mRNAs through their 5′-terminal m^{7}Gppp . . . sequence. This would agree with previous findings of Both *et al.*[13,34] that absence of 7-methylguanosine from the 5′-terminus of certain eukaryotic mRNAs markedly reduces messenger–ribosome complex formation and translation of these messengers and would support the hypothesis of these investigators[34] that the m^{7}Gppp . . . region of mRNA may serve as a specific recognition signal for initiation of eukaryotic protein synthesis.

As indicated above, the affinity of natural mRNA for IF-M$_3$ is probably much greater than that observed with m^{7}G$^{5'}$p. Therefore, the present studies should not be taken to imply that m^{7}G$^{5'}$p represents the complete sequence of mRNA recognised by IF-M$_3$ or that IF-M$_3$ binds solely to this region of mRNA. The reduced but yet significant binding of EMC virus RNA to IF-M$_3$ and the lack of inhibition of this complex by m^{7}G$^{5'}$p would support such a conclusion. These results are also consistent with the observation of Staehelin *et al.*[23], that threefold greater quantities of factor IF-E6 (IF-M$_3$) are required for translation of EMC virus RNA compared with rabbit globin mRNA. In future studies, we hope to better define the process by which IF-M$_3$ interacts with mRNA by comparing the affinity of various ^{3}H-labelled nucleotides for this protein (m^{7}G$^{5'}$p, m^{7}G$^{5'}$pp, m^{7}G$^{5'}$ppp, m^{7}G$^{5'}$pppNp, for example), and to determine the relationship of this event to other mRNA factor interactions which may be involved in the eukaryotic initiation mechanism. Since it has been shown recently that *in vitro* translation of "unblocked" mRNAs, such as satellite tobacco necrosis virus (STNV) RNA[14] and encephalomyocarditis (EMC) RNA L. A. W., E. R. Feman, E. D. Hickey, M. C. Williams, and C. B., unpublished,) is not inhibited by m^{7}G$^{5'}$p, it will also be important to determine whether translation of mRNAs not containing 5′-terminal 7-methylguanosine, proceeds through the same or a different mechanism.

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letters to nature

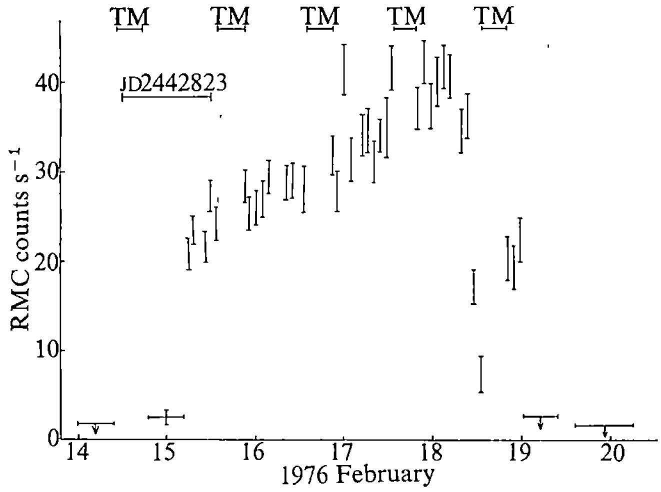
X-ray outburst from Circinus X-1

During observations of the Circinus–Norma region by the rotation modulation collimator (RMC) experiment on board Ariel V, a sudden strong X-ray outburst was detected from Cir X-1. The source appeared on 1976 February 15 at 0500(UT) at an intensity of 20 counts s^{-1}. It gradually brightened to over 40 counts s^{-1} on February 17 at 2200(UT) and then rapidly declined on February 18. Our best position, RA = 229. 19°, Dec = −57.00°, with Error Circle, 0.02° radius (90% confidence), is in excellent agreement with the known position[1] of Cir X-1.

The light curve is shown in Fig. 1. The data have been corrected for instrumental response and source visibility. Data gaps occur whenever the experiment is switched to its 'time' mode of operation, when positional resolution is sacrificed for better time resolution. The data points have a nominal time resolution of ~100 min (1 orbit) except where indicated. The onset of the outburst is extremely rapid. The initial detection in a single orbit gives an intensity of ~20 counts s^{-1}; analysis of the data from the previous orbit gives an upper limit (3σ) of 5 counts s^{-1}. For compatibility with data preceding and succeeding the outburst and the known times when the source was in the field of view, the transition to the high stage must have occurred between 0400 and 0512 (UT) on 1976 February 15. When the data for the preceding 10 h is summed, we obtain a marginal detection at 2.5 ± 0.8 (1σ) counts s^{-1}. Earlier observations give a 3σ upper limit of 1.2 counts s^{-1} for 1976 February 6.5 to February 14.0.

The source showed a rapid decline in intensity following peak brightness, falling to just above the detector threshold in a few hours. After the data gap, however, the source had recovered to half its maximum intensity, remaining at this level for five hours. It subsequently faded to below the detector threshold. Because about six bright sources are in the field of view of the experiment, it is not possible to recover accurately the absolute intensity of Cir X-1 from the time-mode data. The general recovery of the source is, however, qualitatively evident in that data. In the half day period after the source went below the threshold the summed data give an upper limit of 2.5 counts s^{-1} for the time averaged intensity; in any single orbit during this period the upper limit is 7 counts s^{-1}.

Fig. 1 The light curve of Cir X-1 in 2.9–7.6 keV X rays measured by the RMC experiment on Ariel V. The data are corrected for detector response and source visibility. Error bars are ± 1σ and upper limits are 3σ. Time resolution, if not indicated, is ~100 min (1 orbit). For comparison, 1 count s^{-1} is approximately equivalent to 12 Uhuru counts s^{-1}. TM, time mode.



The photon flux is measured by the RMC experiment in three pulse height channels corresponding to energy ranges 2.9–4.3, 4.3–5.9 and 5.9–7.6 keV respectively. This enables us to compute the photon number spectrum for the source, which is plotted in Fig. 2 for maximum X-ray intensity. Throughout the outburst reported here the spectral data can be fitted by a thermal exponential spectrum with kT = 1.7–2.4 keV at a column density of 10^{22} atoms cm^{-2}. But, the data are too insensitive for good evaluation of the absorption term. Unfortunately

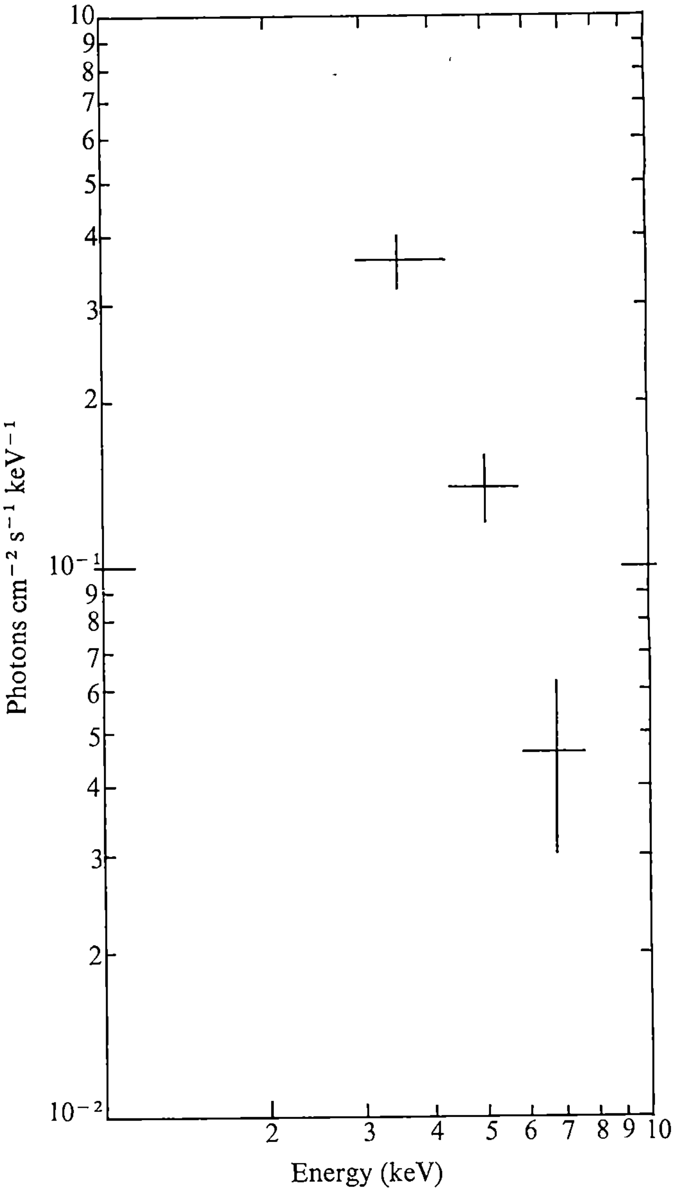
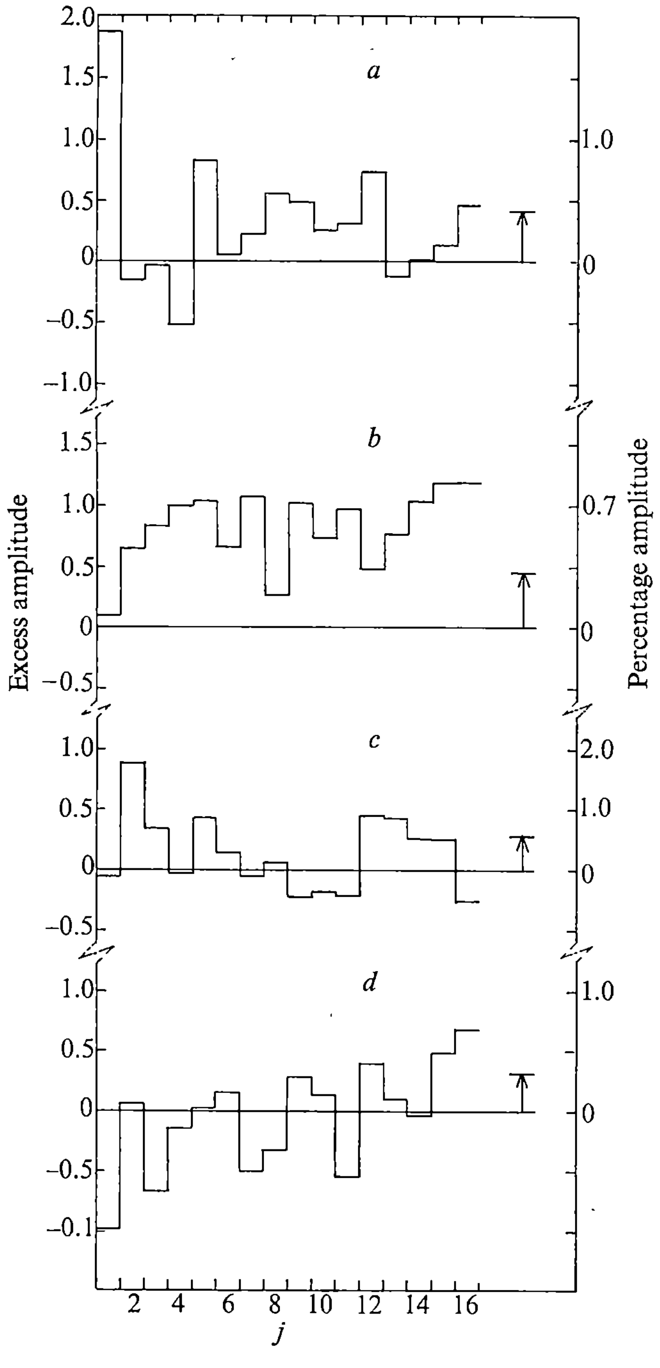


Fig. 2 The X-ray spectrum of Cir X-1 at maximum intensity. Data used are taken from 1976 February 17, 1900(UT) to 1976 February 18 0500(UT). Error bars are ± 1σ.

photon statistics are too poor to allow us to follow spectral changes during the decay phase of the outburst, or to measure a spectrum in the period immediately before the high intensity onset.

In the time mode operations indicated in Fig. 1, counts are simply accumulated in consecutive time bins of 32 s. Sequences of 32 bins were selected which were free of passages through regions of high particle background, and free of occultation of the Sun or the source by the Earth. Any trends in the data were determined by a least squares analysis fitting a polynomial, degree 3, and subtracted out, except for the pre-outburst data. Power spectra were then obtained for each sequence and summed. The results are summarised in Fig. 3. The abscissa is: $(P_j - \langle P\rangle)/\langle P\rangle$ where P_j is the amplitude in the jth frequency bin and $\langle P\rangle$ is that expected from counting statistics from a constant source; $\langle\sigma_p\rangle$ is the expected s.d. from counting statistics.

Fig. 3 Mean power spectra measured by the RMC experiment on Ariel V. The abscissa is the excess amplitude measured in units of the expected amplitude; the corresponding percentage amplitude is indicated. The ordinate, j, is frequency bin number, bin 1 corresponds to 0.00098 Hz, bin 16 corresponds to 0.0156 Hz. *a*, Six sequences of 32 time bins measured on 1976 February 13–14; *b*, nine sequences of 32 time bins measured on 1976 February 15–17; *c*, three sequences of 32 time bins measured on 1976 February 18; *d*, ten sequences of 32 time bins measured on 1976 February 20–21. Arrow on abscissa gives the s.d. $\langle\sigma_p\rangle$.



The pre-outburst mean power spectrum shows a non-random power distribution which we attribute to other sources in our field of view. During the outburst from Cir X-1, excess power density is measured at all frequencies with no single frequency-bin outstanding. The power density in the decay phase of the outburst is much less and approaches that measured in data since the outburst.

The first definite observation[2] of Cir X-1 was made in 1969. Previous observations give a conflicting view of the sky in that region and source confusion must be assumed. Since then many observations[1,3–7] of the source show it predominantly at a low intensity, with occasional outbursts to a higher level, with rapid, erratic changes in its behaviour, variable on time scales down to ⩽ 1 s. The model of Cir X-1 as a young runaway binary in a highly elliptical, long period orbit[9], would predict high X-ray emission from Cir X-1 near periastron. The data of Baity *et al.*[4], and Davison and Tuohy[7] suggest an interval of ~220 d between times of high X-ray emission. Since the Copernicus observation[7], SAS-3 has observed Cir X-1 (ref. 8) and our report further extends the base line. Our data give a well determined time at which an outburst occurred, and record a complete cycle of increased emission. The behaviour is consistent with an outburst of high intensity every 220–250 d, but consideration of the reported times of previous outbursts suggests that the phenomenon is not strictly periodic but irregular. Our data are not good enough to confirm any softening of the spectrum with enhanced emission[1,4], or to give any new information on changes in absorption during the event[2]. The softness of the spectrum does not conflict with the suggestion that the long term intensity variations of Cir X-1 are due to low energy X rays[4].

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The infrared spectrum of Nova Cygni 1975

Grasdalen and Joyce[1] have observed emission lines from Nova Cygni 1975 in the 2 and 3 μm spectral regions. In mid-September 1975, the dominant spectral features were recombination lines of hydrogen. Grasdalen and Joyce noted the presence of a number of additional lines in a later spectrum and identified these with forbidden transitions in highly ionised forms of Mg, Ca, Al, and Si. The prerequisite for formation of such coronal lines is an

Table 1 Infrared line identifications

λ_{obs} (μm)	Peak flux (Jy) September 12, 1975	Peak flux (Jy) October 27, 1975	λ_{pred} (μm)	
1.90	>30	>1.8	1.909	He I 4^1D–3^1P^0
1.943	33	1.8	1.945	H Brδ
2.06	30	1.7	2.060	He I 2^1P^0–2^1S
2.11	17	1.0	2.114	He I 4^1S–2^1P^0
2.167	52	2.9	2.165	H Brγ
2.86	28	1.6	2.87	H Pfζ
3.06	30	1.7	3.04	H Pfε
3.27	32	1.8	3.30	H Pfδ, He I 5^1P^0–4^1S
3.50	27	1.5	unidentified	
3.73	37	2.1	3.74	H Pfγ
4.02	100	5.6	4.05	H Brα, He I 5^1P^0–4^1D
1.959		2.0	1.954	He I 4^3P^0–3^3D
2.040		1.9	2.042	He I 6^3P^0–4^3S
2.32		0.3	2.321	He I 13^3P^0–5^3S
2.474		3.8	2.473	He I 6^3D–4^3P^0
2.879		2.2	2.854	He I 5^3P^0–4^3S
3.021		8.2	3.033	He I 10^3F^0–5^3D
3.18		0.8		
3.661		3.9	3 678	He I 13^3P^0–6^3S
3.72		3.9	3.703	He I 5^3D–4^3P^0
3.92		2.0	3.910	He I 14^3D–6^3P^0

emitting region of low density and extremely high temperature, $T \sim 10^6$ K, which, moreover, must span a considerable range of temperature in order for species such as Al V, VI, VIII and IX to exist simultaneously. This would seem to preclude an isothermal shock as the mechanism responsible for a high temperature region in Nova Cygni. We propose a more conservative identification of these new lines, namely that they are all lines of He I with one possible exception. Furthermore, their sudden appearance and increase in relative intensity are in accord with somewhat more temperate conditions ranging from $T \sim 10^4$ K in September 1975 to $T \sim 10^5$ K in October. This identification is also more consistent with the known behaviour of very fast novae, which rarely exhibit optical coronal emission lines[2].

Table 1 lists the wavelengths of the features observed by Grasdalen and Joyce and our suggested identifications. The observed wavelengths are from the table of Grasdalen and Joyce or are estimated from their figures. The peak fluxes have been estimated from the published spectra and from the scaling procedure used in the original analysis. These fluxes are presented to illustrate the change in relative fluxes between September 12 and October 27; they should not be taken literally. The predicted wavelengths have been taken from standard references[3,4].

The strongest lines in the September spectrum were correctly identified by Grasdalen and Joyce as Brackett and Pfund series lines of hydrogen. As many as five lines of He I also appear, although two are blended with stronger H lines. We suggest that these are not recombination lines, but rather the result of pumping of metastable He I (2^1S) by the very broad, very strong hydrogen Balmer continuum short of 3,646 Å. This pumping occurs through the transition 2^1S–5^1P^0 at 3,614 Å, and the 5^1P^0 state decays through a cascade which includes all the singlet transitions listed in Table 1. Table 2 lists additional lines in the visible and near infrared which would also appear as part of this fluorescence process, and which should share the intensity variation exhibited by the infrared lines. The absence of detectable triplet transitions in He I (which should dominate the recombination spectrum if He were ionised to the same degree as H) is consistent with a collision-excited plasma with $T \sim 10^4$ K in September.

Table 2 He I lines excited by Balmer continuum

Transition	Wavelength (Å)
4^1S–2^1P^0	5,047
4^1D–2^1P^0	4,921
5^1P^0–3^1S	11,013
5^1P^0–3^1D	12,756
3^1P^0–2^1S	5,015
3^1S–2^1P^0	7,281
3^1D–2^1P^0	6,678

According to our interpretation, the October spectrum shows strong He I recombination lines. The agreement between observed and predicted wavelengths is at least as good as that found by Grasdalen and Joyce for the coronal ions, and all the more striking since only one species is involved. The relative intensities of the suggested He I lines are in harmony with transition probabilities[4] and qualitative expectations from recombination theory. Note that the He I lines are as strong as the H lines in the October spectrum. This can occur in a plasma at $T \sim 10^5$ K in which H and He are present in normal cosmic abundance (10–15% He by number) and are both singly ionised. At such temperatures, He recombines nearly ten times faster than H because of dielectronic recombination and should thus exhibit an appropriately stronger recombination cascade. A temperature of $T \sim 10^5$ K is further supported by the appearance of strong emission from the N III 4,640 Å blend in October because at $T = 10^{4.9}$ N III becomes the dominant stage of nitrogen[5]. This interpretation of the infrared spectrum of Nova Cygni is clearly subject to further observational tests through examination of He I lines in visible spectra obtained during the same time period. Not only the physical evolution of the emitting region, but also the helium abundance may be determined from such studies A more detailed analysis of the recombination spectra in Nova Cygni 1975 will be published elsewhere.

The existence of regions in Nova Cygni with $T \sim 10^5$ K seems physically reasonable. Although the optical decline was probably paralleled by a true decrease in luminosity, some energy source must have remained to provide for the substantial optical and infrared flux. From the observations reported by Gallagher and Ney[6], the luminosity can be estimated at $\sim 10^4 L_\odot$ in late October. Both theoretical and empirical arguments suggest that, by this stage, the nova remnant would have a small radius and correspondingly high effective temperature (G. T. Bath and G. Shaviv, and J. S. Gallagher and S. G. Starrfield, unpublished). The He I emission might then originate in a dense, hot medium near the underlying star.

Finally, we call attention to the unidentified line at 3.50 μm and to the line at 3.18 μm, which appear not to be He I lines. The latter may indeed be [Ca IV] as suggested by Grasdalen and Joyce since Ca exists predominantly in this stage of ionisation at our inferred temperature.

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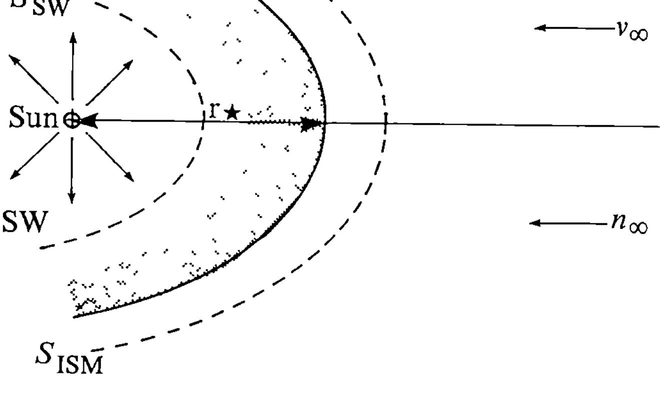
Can cosmic clouds cause climatic catastrophes?

SEVERAL authors[1–3] have discussed whether past immersion of the Solar System in dense interstellar matter (ISM) might have left observable imprints on the Earth. Accretion cannot affect the solar constant significantly unless the surrounding ISM attains densities of 10^5–10^7 cm^{-3} (refs 2 and 3). We demonstrate here, however, that a much more modest ISM density of 10^2–10^3 cm^{-3} (a value typical of dense interstellar clouds in spiral arms) would prevent the solar wind from reaching the Earth, and propose that such a change in the Earth's space environment may trigger drastic climatic changes.

Suppose that the Sun moves with velocity v_∞ through ISM of density n_∞ and temperature T_∞. The solar wind can be envisaged as a supersonic radial flux of fully ionised particles with density n_E and velocity v_E at the Earth ($r = 1$ AU). If there were no mixing or interpenetration between solar wind and ISM, there would be a contact discontinuity with the rough shape shown in Fig. 1, located at a distance r_* from the Sun in the 'upstream' direction. (In practice, the SW may be shocked before reaching r_*; and the ISM, if its properties were sufficiently 'fluid-like', might pass through a shock outside r_*.)

Fig. 1 Schematic depiction of the termination of the solar wind (SW) by the interstellar medium (ISM). With respect to the Sun, the ISM has streaming speed v_∞, density n_∞, at $r \to \infty$. The termination radius r_* is found by balancing ram pressure on either side of an imaginary contact discontinuity C. If the ISM is largely neutral, r_* is the outer boundary of a region of interpenetration (shaded). In the text, we show that the value of n_∞ necessary to make r_* smaller than 1 AU never exceeds $n_\infty \simeq 100\,(v_\infty \simeq 10$ km s^{-1}) cm^{-3} by more than a factor of 2 or 3, regardless of the ionisation level of the ISM. Allowance for possible shock discontinuities S_{SW} and S_{ISM} would reduce the required n_∞. Clouds populating the spiral arms of the Galaxy may have sufficient densities to prevent the solar wind from reaching the Earth.



In the idealised case shown in Fig. 1, r_* has the value at which the 'ram pressure' $\propto n_E v_E^2/r_*^2$ of the solar wind balances the pressure of the infalling ISM. We can estimate this as follows. The speed of a typical ISM particle at distance r is

$$v(r) \simeq \left(v_\infty^2 + v_{th}^2 + \frac{v_{esc}^2}{r_{AU}}\right)^{\frac{1}{2}}$$

where $v_{esc} \simeq 42$ km s^{-1} is the escape speed from the Sun at 1 AU. In most cases of interest, v_∞ will be greater than the thermal velocity $v_{th} \simeq (T_\infty/100)^{\frac{1}{2}}$ km s^{-1}; and we therefore neglect v_{th} to keep our discussion short. If the mean free path in the ISM is so large that, until reaching r_*, each particle follows an independent hyperbolic orbit under the influence of the Sun's gravity, then the density at a distance r ahead of the Sun is $n(r) \simeq n_\infty \; v(r)/v_\infty$ due to 'gravitational focusing'. Defining r_* by the equality $n(r_*)\,[v(r_*)]^2 = n_E v_E^2/r_*^2$, we obtain

$$r_* \propto \begin{cases} n_\infty^{-1/2} v_\infty^{-1} & \left[r_* \gtrsim \left(\dfrac{v_{esc}}{v_\infty}\right)^2\right] \quad (1a) \\[2ex] n_\infty^{-2} v_\infty^{2} & \left[r_* \lesssim \left(\dfrac{v_{esc}}{v_\infty}\right)^2\right] \quad (1b) \end{cases}$$

Once n_∞ becomes large enough to bring r_* below $(v_{esc}/v_\infty)^2$ AU, the gravitational focusing causes r_* to depend much more sensitively on n_∞. When the inflow of the ISM is fluid-like and standard accretion theory can be used, the density enhancement goes as $(v/v_\infty)^3$ rather than just as (v/v_∞), so the dependence is more sensitive still. Taking $v_E = 450$ km s^{-1} and $n_E \simeq 5$ cm^{-3}, equation. (1*b*) implies that r_* would lie within 1 AU when $n_\infty \gtrsim 100\,(v_{esc}/10$ km s^{-1}) cm^{-3}. Allowance for fluid-like behaviour of the ISM would lower the required n_∞ further, but we note that it is in any case comparable with 'typical' interstellar cloud densities. (If the ISM outside r_* behaved as a fluid, we would obtain an even more drastic result than equation (1*b*): r_* would be forced down to zero (the solar wind would be entirely 'snuffed out') whenever r_* were forced within a value $\sim (v_{esc}/v_\infty)^2$ AU, because within that radius the ram pressure provided by radial accretion is $\propto r^{-5/2}$, which is steeper than the r-dependence of the solar wind pressure.

When the ISM is mainly neutral, hydrogen is acted on by radiation pressure from the solar Lyman α (refs 4 and 5). This yields a repulsive force comparable to the Sun's gravitational attraction, thereby reducing the gravitational focusing. Significant photoionisation of neutral interstellar hydrogen (H I) would occur within a few AU; and any penetration of ionised ISM into the solar wind would be prevented by plasma instabilities and the transverse solar wind magnetic field. If some ISM encounters the solar wind while still neutral, the contact discontinuity will be replaced by a region of interpenetration whose thickness is governed by the length scale for ionising the remaining neutral particles. Once ionised, such particles rapidly join the bulk solar wind flow. This flow will be slowed down by charge exchange reactions in the interpenetration region[4].

The character of the transition region at $r \lesssim r_*$ is rather complicated; and effects such as radiation pressure and charge exchange would modify the simple formulae we have given for r_*. We find, however, that the ISM density required to reduce r_* below 1 AU exceeds that inferred from equation (1) by a factor of no more than 2 or 3 (M. Begelman, in preparation); moreover, the corrections for fluid-like behaviour outside r_* (which become more important for high n_∞) tend to reduce the required density. Of course r_* is measured along the line of stagnation. The termination distance in the plane containing the Sun and perpendicular to the line of stagnation is, however, unlikely to be much more than $\sim 2r_*$. Therefore, if $r_* \lesssim 0.5$ AU the Earth will spend part of each year out of reach of the solar wind. If the solar velocity relative to the ISM makes a large angle with the plane of the ecliptic, the Earth may be entirely cut off from the solar wind.

The ISM attains densities $\gtrsim 10^2$ cm^{-3} in H I clouds, in regions of star formation (for example the Orion Nebula)[6,7], and in thin sheets swept up by supernova remnants or by the winds of O or B stars[8,9]. These dense regions would be concentrated in spiral arms. The Solar System traverses an arm every $(1-3)\times10^8$ yr (this figure being uncertain because we know neither the speed of the spiral wave, nor how regular or long-lived the spiral pattern is); and during each traversal its orbit may encounter several such dense regions. McCrea[2,3] has suggested that there may be a causal connection between spiral arm traversals and the quasi-periodic epochs of glaciation inferred from the geological record. He tentatively relates the Quaternary glaciation to the Sun's passage into the Orion Arm. But the specific mechanism that he proposed—an alteration of the solar constant caused by accretion—requires an interstellar cloud of density 10^5–10^7 cm^{-3}, and such clouds are so compact and unusual that the Sun would have little chance of encountering even one.

There is now an impressive array of evidence suggesting that solar activity influences the Earth's climate through the effects of the solar wind. Immersion in the less extreme and more widespread ISM regions with density 10^2–10^3 cm^{-3} can prevent the solar wind from reaching the Earth. This circumstance therefore suggests a possible physical basis for McCrea's conjectured link between spiral arms and terrestrial climate which obviates the need for the extreme ISM densities required by his specific accretion mechanism.

Exclusion of the Earth from the 'heliosphere' might have four major effects: (1) If the ISM were largely neutral at 1 AU, the Earth would be bathed in an enhanced background of cool neutral material: this material could penetrate the magnetosphere and join the upper ionosphere, perhaps significantly changing its composition over periods of $\gtrsim 10^6$ yr. If charge exchange reactions were important in the interpenetration zone, neutral hydrogen would flow past the Earth at solar wind speeds. These particles would also penetrate the magnetosphere, and probably cause ionisation by collisions in the ionosphere. (2) Ions in the vicinity of the Earth would typically have speeds much lower than those of typical solar wind particles. This would affect the rate at which they could be trapped by the magnetosphere, as well as the rate at which trapped particles could be funnelled into the atmosphere over the polar regions. (3) In the absence of solar wind modulation, an enhanced flux of low energy interstellar cosmic rays would reach the Earth. (4) The Earth's magnetosphere would be isolated from the solar magnetosphere. This would seriously inhibit bursts of energetic particles, emitted in such events as solar flares, from reaching the Earth, and is perhaps the most crucial effect.

There is no theory which predicts what effects any of these changes might have on the climate. King[10,11] and others[12] have found correlations between weather and local short term variations in the geomagnetic field, and direct correlations have been found between atmospheric circulation patterns and crossings of solar wind sector boundaries[13]. These results, suggesting that the lower atmosphere somehow reacts sensitively to the solar wind, are consistent with studies of long term correlations, which take into account migration of the Earth's magnetic poles and polarity reversals. Wollin *et. al*[12,14] have found that magnetic intensity and temperature are inversely correlated.

One mechanism which has been suggested for explaining these apparent correlations asserts that the lower atmosphere is affected by energetic particles emanating from the Sun in flares. These particles could change the ionisation balance in the lower atmosphere, affecting among other things its conductivity[10] and ozone content[10,15]. Access of particles with a given energy is strongly regulated by the Earth's intrinsic magnetic field, as well as the coupling between the solar wind and the magnetosphere. Thus, the correlations cited above could be rationalised according to this picture. Replacing the solar wind by interstellar medium might then have the same effect (a reduction in the flux of solar particles reaching the Earth) as making the Earth's magnetic field very strong. If terrestrial temperature is indeed correlated with energetic particle flux, then shutting off the solar wind may be just what it takes to trigger an ice age.

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Possibility of detecting magnetospheric radio bursts from Uranus and Neptune

EARTH, Jupiter and Saturn are sources of intense but sporadic bursts of electromagnetic radiation which we call magnetospheric radio bursts (MRBs). The Earth's MRBs are observed at kilometric wavelengths, with a power flux spectral peak near 200–300 kHz, a bandwidth roughly half the peak frequency, and a high frequency cutoff near but below the electron gyrofrequency corresponding to the polar surface magnetic field. The Earth's MRBs last a few minutes. Jupiter's MRBs occur at decametric wavelengths, with a power flux spectral peak near 7–8 MHz, a bandwidth again roughly half the peak frequency, and a high frequency cutoff below the polar surface electron gyrofrequency; Jupiter's MRBs also last a few minutes. Saturn's MRBs are observed at hectometric wavelengths, with a power flux spectral peak near 1 MHz and a bandwidth of roughly half the peak frequency, and again last a few minutes. The similarities in the power flux spectra together with the burst occurrence patterns suggest a common physical origin for the MRBs of all three planets. Perhaps the common mechanism is noise amplification by field aligned currents since Gurnett[1] has shown that Earth's MRBs are associated with bright auroral arcs which involve intense field aligned currents. Field aligned currents result from the interaction of the solar wind with the magnetosphere and should be a general feature of the interaction between the solar wind and a planetary magnetosphere (Vasyliunas[2] and Kennel[3]). If MRBs are ultimately produced by the solar wind–magnetosphere interaction, their total radiated power might scale as the solar wind power input into the magnetosphere Kaiser and Stone[4] have suggested the frequency of emission scales as the polar magnetic field strength of the planet. Here, we scale the intensity of MRBs to the solar wind

Table 1 Values for quantities used in estimating the detectability of MRBs are listed for each of the planets

	B_p (gauss)	B_{Bode} (gauss)	$\frac{P_N b_N}{\dot{W}_p}$	$\dot{W}_p$ (W)	Peak frequency (MHz)	Peak power flux at Earth (W m^{-2} Hz)	Total radiated power (W)
Earth	0.5†	0.5	$\sim 10^{-3}$	5×10^{11}	0.25†	—	5×10^{8}
Jupiter	14–18†	14	3×10^{-2}	5×10^{13}	8†	2×10^{-19}†	1.5×10^{12}
Saturn	2*	4.8	5×10^{-2}	5×10^{12}	1.1†	5×10^{-20}†	3×10^{11}
Uranus	—	1.6	5×10^{-2}*	4×10^{11}	0.85	10^{-21}	2×10^{10}
Neptune	—	1.3	5×10^{-2}*	1.5×10^{11}	0.7	2×10^{-22}	8×10^{9}

†Measured.
*Inferred from scaling law.

The values of peak frequency, power flux and total radiated power for Uranus and Neptune are calculated assuming the Bode's law value of the polar field strength. The total radiated power is based on the assumption of isotropic hemispherical radiation.

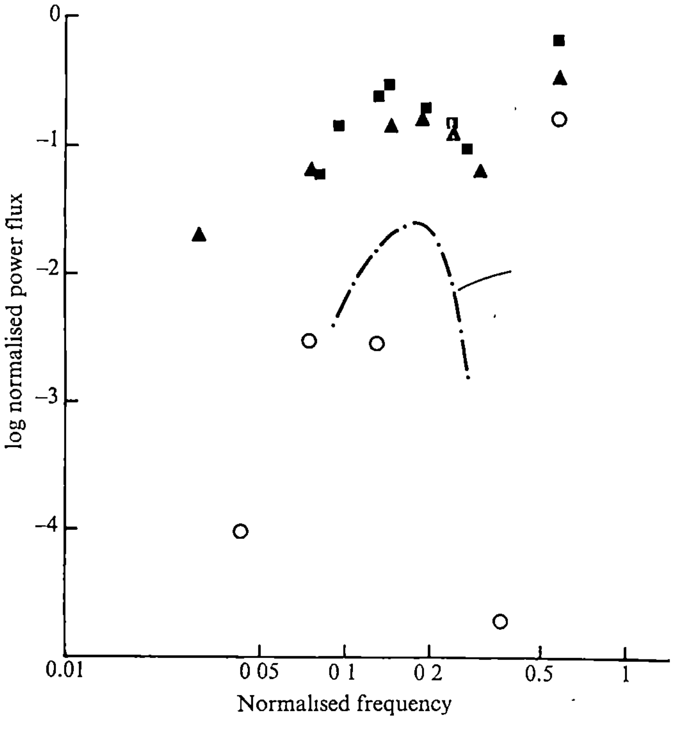
input and the frequency of emission to the polar field strength to estimate the possibility of detecting MRBs from Uranus and Neptune.

For determining the detectability of MRBs, we hope to find a scaling law that relates the ratio of power radiated in MRBs to the solar wind input for Earth, Jupiter and Saturn. The total power radiated in MRBs, P_p, relative to the solar wind dissipation rate, $\dot{W}_p$, can be found by integrating the observed power flux, P_p', over frequency, ω, and multiplying by the area, A, over which the noise is radiated

$$\frac{P_p}{\dot{W}_p} = \frac{A}{\dot{W}_p}\int P_p'\,d\omega = \int P_p'\,\frac{A\Omega_p}{\dot{W}_p}\,\frac{d\omega}{\Omega_p} = \int \overline{P_p}'\,d\overline{\omega} \quad (1)$$

where $\overline{\omega}$ is the frequency normalised to the electron gyrofrequency corresponding to the surface polar field strength, Ω_p, and $\overline{P_p}'$ is the power flux normalised by the quantity $A\Omega_p/\dot{W}_p$.

Fig. 1 The normalised observed power flux spectra, $\overline{P_p}'$, (see equation (1)) of very intense radio bursts from Earth, ○ (Gurnett[1]), Jupiter, ▲, and Saturn, ■ (Brown[10]) are plotted as functions of normalised frequency. Saturn's field is predicted by using the magnetic Bode's law. All spectra peak between 10–20% of the surface electron gyrofrequency and have similar bandwidths, but Jupiter and Saturn produce more power relative to the solar wind input than does the Earth. For completeness we have also sketched (· – · – ·) a spectrum based on properties of the Earth's MRBs described by Kaiser and Stone[4], whose experiment had many more channels in the frequency range of interest than did Gurnett's[1], but not enough dynamic range to measure the most intense MRBs of the Earth.



To find the area, A, we assume the radiation is isotropic over a hemisphere. In doing this, we overestimate the total power radiated in MRBs since Earth's MRBs are known to be somewhat beamed (Gurnett[1]) while the beaming of Jupiter's high frequency decametric radiation is well known (Warwick[5]). The beaming of radiation at frequencies near the peak power flux is, however, far less pronounced (Dulk and Clark[6]) than at higher frequencies near the electron cyclotron frequency where the power flux is much lower. Since we cannot systematically estimate the beaming factor, we choose to assume isotropic hemispherical radiation for the purpose of scaling.

The solar wind power dissipation can be estimated from the work done by the magnetopause currents at the nose of the magnetosphere in the motional e.m.f. of the solar wind. Using standard theory to define solar wind parameters, Kennel[7] estimated that the power dissipation $\dot{W}_p$ into a given planetary magnetosphere scales to that at earth $\dot{W}_E$ as

$$\frac{\dot{W}_p}{\dot{W}_E} = \left(\frac{M_p}{M_E}\right)^{2/3} r^{-4/3} = \left(\frac{R}{R_E}\right)^{2}\left(\frac{B_p}{B_E}\right)^{2/3} r^{-4/3} \quad (2)$$

where $M = B_p R^3$ is the magnetic dipole moment, B_p is the surface magnetic field, R is the planet's radius, and r its heliocentric distance in AU.

Kaiser and Stone[4] point out that Jupiter's observed polar field strength can be correctly predicted from the observed properties of the MRBs of Earth and Jupiter by assuming that the maximum (cutoff) frequencies of MRBs are the surface electron gyrofrequencies and that the maximum and peak frequencies of MRBs scale linearly with B_p. Using the observed properties of SMRB and the same assumptions they estimate a polar field strength of 2 gauss for Saturn. Saturn's estimated field strength also agrees within a factor 2 of the 'magnetic Bode's law'[8] estimate (used by many authors as a guide to estimate the magnetic fields of planets where observations were not available), which assumes that a planet's dipole moment M scales as its rotational angular momentum L. While there is little theoretical justification for the magnetic Bode's law, Hill and Michel[9] have pointed out that it is surprisingly well obeyed by the objects in the Solar System, in the sense that the spread in M/L is much less than the spread in L.

Using measured values of B_E and B_J and the Bode's law estimate for B_S, we plot in Fig. 1 the normalised power flux spectra $\overline{P_p}'$ of the MRBs of Jupiter and Saturn measured by Brown[10] and a spectrum of Earth's MRBs measured by Gurnett[1]. We can approximate the integration of the power flux in equation (1) by taking the peak normalised power flux, P_N, and multiplying by b_N, the bandwidth in normalised frequency

$$P_p/\dot{W}_p \simeq P_N\,b_N \quad (3)$$

Using equation (3), we see from Fig. 1 that Jupiter and Saturn may radiate 1–5% of the solar wind energy input into the magnetosphere while Earth may radiate only ~0.1%. These percentages must be viewed with caution, however, since the Earth's MRBs shown may not represent the most intense

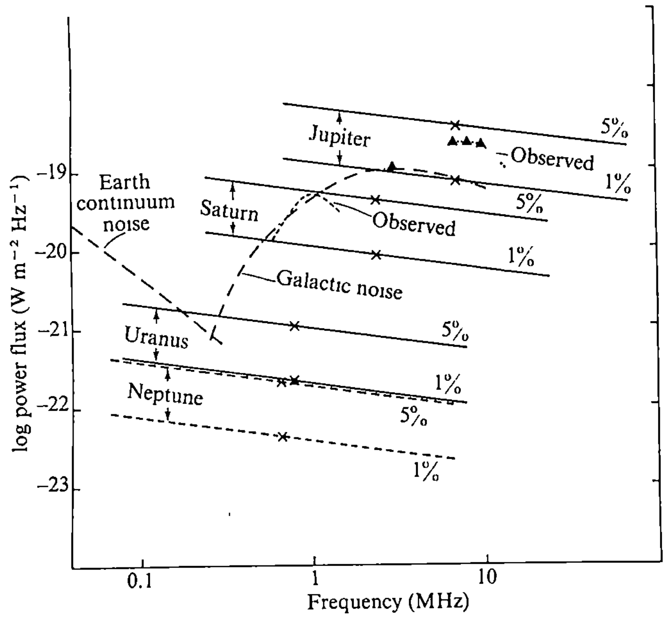


Fig. 2 The locus of the peak in the MRB power flux spectrum at Earth is shown as a function of surface magnetic field strength for efficiencies of 1 and 5% conversion of solar wind power input into the planet's magnetosphere to the most intense MRBs. The surface field strength ranges from 0.1 to 10 times the magnetic Bode's law value, which is indicated by a cross for each planet. The peak frequency is assumed to vary linearly with surface field strength, B_p. The bandwidth of MRBs is taken as half the peak frequency, and since the solar wind power input varies as $B_p^{2/3}$, the peak power varies as $B_p^{-1/3}$. The peak power flux of MRBs from Uranus and Neptune is conjectured to lie between the 1–5% lines. The noise level of a short electric dipole in Earth orbit at $30R_E$ is indicated by –·–·–. Both Uranus and Neptune are near the noise levels, but MRBs from both planets may be detectable from Earth orbit, especially if the surface field strengths are below the magnetic Bode's law estimate.

events; the total number of observed Saturn's MRBs is small, and the MRB spectrum of Jupiter shown is a composite obtained by averaging the intensities of the most intense events. The intensity will also depend on the beaming of the radiation and may further be affected by factors we have not discussed, such as Io-like moons inside the magnetosphere. The close similarity of the MRB normalised spectra of Jupiter and Saturn, and the similar, but lower, MRB normalised spectra of the Earth, suggest, however, that the scaling of MRB power to the solar wind–magnetosphere dissipation power is a reasonable hypothesis.

We now estimate the properties of MRBs from Uranus and Neptune by assuming a 1–5% conversion efficiency, $W_E = 5\times10^{11}$ W, a bandwidth of half the peak frequency, and that dipole moments of Uranus and Neptune roughly obey the magnetic Bode's law. In Fig. 2 we plot the peak power flux for 1 and 5% efficiency for Uranus, Neptune, Saturn and Jupiter, assuming various values of B_p. The Bode's law estimates are indicated by a cross. We have superposed curves of Earth interference at $30R_E$ and galactic noise for a short dipole antenna. In Table 1 we list the parameters used in estimating the detectability of MRBs.

The noise curves probably give a too pessimistic impression concerning the detection of MRBs for Uranus and Neptune, since knowing the location of a possible bursty source and using an antenna more directional than a short dipole may facilitate detection below the noise level. Figure 2 also indicates that the situation only improves away from Earth, since the Earth continuum noise is reduced and the power fluxes from Uranus and Neptune should increase as the square of the distance to the planet. We therefore suggest that detection of magnetospheric radio bursts from Uranus and Neptune might be a reasonable cruise mode radio astronomy objective on future missions to the outer Solar System.

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Dirac's continuous creation cosmology and the temperature of the Earth

DIRAC[1,2] has modified his earlier cosmological model based on the large number hypothesis[3,4], that the observed approximate equalities

$$\frac{e^2}{Gm^2} \simeq \frac{mc^3}{He^2} \simeq \left(\frac{\rho c^3}{mH^3}\right)^{\frac{1}{2}} \simeq 10^{39} \qquad (1)$$

are not accidental but causal—even though we are as yet unaware of the nature of the relationship between cosmology and local physics (e is the electronic charge, G the gravitational constant, c the velocity of light, ρ the mean density of the Universe, H Hubble's constant and m an atomic mass). In the earlier version, Dirac assumed mass conservation and that equalities (1) held for all time, thus

$$R(t) \propto t^{1/3};\, H \propto \frac{1}{3t};\, G \propto \frac{1}{t} \qquad (2)$$

where $R(t)$ is the cosmological scale factor, $H = \dot{R}/R$. In the modified version, Dirac argues (unconvincingly in my view) that $R(t) \propto t$, in which case

$$G \propto \frac{1}{t};\, H \propto \frac{1}{t};\, \rho = \frac{\rho_0}{t^3} \propto \frac{1}{t};\, \rho_0 \propto t^2$$

that is, matter is continuously created such that $\rho_0 \propto t^2$ where t is the age of the Universe. This newly created matter could be produced uniformly throughout space, or locally in proportion to the amount and composition of matter already present. Dirac prefers the second alternative, but we shall show that this leads to an unacceptable past temperature for the Earth.

What are the properties of the newly created matter; is it at rest relative to the cosmological background, or is it created at rest relative to the local matter? If the creation field is a scalar we would expect the former, but it could be a vector or tensor field and lead to the latter. (I would like to thank the referee for emphasising this second possibility.) In the absence of any way of deciding between these two possibilities we consider both; in either case the mass of any body, $M \propto t^2$.

The luminosity of a star of solar mass satisfies the approximate proportionality

$$L \propto G^7M^5 \propto t^3 \qquad (3)$$

and increases with time. On the other hand the orbit of the Earth with mass $m(t)$ satisfies the equation

$$\frac{\mathrm{d}}{\mathrm{d}t}(m\dot{\mathbf{r}}) = -\frac{GMm}{r^2}\,\hat{\mathbf{r}} \text{ (creation at rest)} \tag{4}$$

or

$$m\frac{\mathrm{d}\dot{\mathbf{r}}}{\mathrm{d}t} = -\frac{GMm}{r^2}\,\hat{\mathbf{r}} \text{ (comoving creation)} \tag{4a}$$

These equations have an angular momentum integral

$$mr^2\dot{\theta} = A \text{ (a constant)} \tag{5}$$

or

$$r^2\dot{\theta} = h \text{ (a constant)} \tag{5a}$$

and therefore the reciprocal radius, u, satisfies the orbit equation

$$\frac{\mathrm{d}^2u}{\mathrm{d}\theta^2}+u = \frac{GMm^2}{A^2} \tag{6}$$

or

$$\frac{\mathrm{d}^2u}{\mathrm{d}\theta^2}+u = \frac{GM}{h^2} \tag{6a}$$

Thus the radius R of the orbit varies in time

$$R \propto \frac{A^2}{GMm^2} \propto t^5 \tag{7}$$

or

$$R \propto \frac{h^2}{GM} \propto t \tag{7a}$$

The orbit spirals inwards in both cases. Combining equations (3) and (7) (or (7a)) we find the equilibrium temperature of the Earth

$$T_{\mathrm{E}} \propto \left(\frac{L}{R^2}\right)^{1/4} \propto (G^9M^{11})^{1/4} \propto t^{13/4} \tag{8}$$

or

$$\propto (G^9M^7)^{1/4} \propto t^{5/4} \tag{8a}$$

Now the inverse Hubble constant has a value of about 2×10^{10} yr which in this cosmological model $R(t) \propto t$ is the age of the Universe; since the Earth has an age of $\sim t_{\mathrm{E}} = 5\times10^9$ yr, the temperature has increased from

118 K ($t_{\mathrm{E}} = 0$) to 177 K ($t_{\mathrm{E}} = 3\times10^9$ yr) to 300 K
(creation at rest)

209 K ($t_{\mathrm{E}} = 0$) to 245 K ($t_{\mathrm{E}} = 3\times10^9$ yr) to 300 K
(comoving creation)

The Earth was substantially cooler in the past than today. If our understanding of climatology is at all correct, this would imply that the Earth was covered with ice and such an ice cover would not have melted even when the equilibrium temperature reached its present value, because of the high albedo of an ice-covered Earth. The existence of life some 3×10^9 yr ago would apparently rule out these cosmological models.

Uniform creation would leave the masses of the Sun and Earth effectively constant so that with $G \propto 1/t$

$$T_{\mathrm{E}} \propto \left[\frac{1}{t}\right]^{9/4} \tag{9}$$

Since in this model $R(t) \propto t$, $H = 1/t$, the age of the Universe is 2×10^{10} yr, and from equation (9) it follows, therefore, that some 3×10^9 yr ago the temperature at the Earth would have been

$$T_{\mathrm{E}} = \left(\frac{20}{17}\right)^{9/4} 300\,\mathrm{K} = 412\,\mathrm{K} \tag{10}$$

too hot for life to have developed, unless the increased solar flux does not lead to a corresponding increase in the Earth's temperature because of the increased albedo of a cloud-covered Earth.

A way out of this problem is to have

$$T_{\mathrm{E}} \propto (G^9M^{11})^{1/4} \tag{11}$$

a slowly varying function of time; for example, since equation (1) gives $G \propto 1/t$, if we take $M \propto t$ then $T_{\mathrm{E}} \propto t^{1/2}$, slightly cooler in the past but not uncomfortably so. This then requires $\rho_0 \propto t$ and thus

$$R(t) \propto t^{2/3} \tag{12}$$

the Einstein–de Sitter cosmological solution, only now there is creation of matter proportional to the age of the Universe. For such a model the age of the Universe is $2/3H$ or 1.4×10^{10} yr so that 3×10^9 yr ago, the Earth's temperature would be $\sim$ 15% cooler, or 45 K below the present value, which is within the error of uncertainty of climatic models.

While such a model, or a similar one, may not be inconsistent with the observed data, it is somewhat *ad hoc*.

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Variable *G*: a solution to the missing mass problem

DIRAC[1] suggested in 1937 that the gravitational 'constant' G might vary with time. This idea has since been used in several cosmologies, particularly those of Hoyle and Narlikar[2] and those invoking the Brans-Dicke theory of gravity[3]. Limits to $\dot{G}$ have been suggested by Barnothy and Tinsley[4] from the observed form of the redshift–magnitude relation and by Dearborn and Schramm[5] from the existence of clusters. Nevertheless, Van Flandern[6] has provided substantial observational support for a secular decrease in G such that $\dot{G}/G = -(7.5\pm2.7)\times10^{-11}$

yr^{-1}, and this is supported by Morrison and Ward's[7] work on the transits of Mercury. The idea deserves to be explored further, pragmatically in non-cosmological contexts, but we are here primarily concerned with the explanation it affords for the 'missing mass' paradox.

Let us begin with the orbits of two point masses. It is easy to verify numerically Vinti's[6,8] relationships

$$\frac{\dot{G}}{G} = -\frac{\dot{a}}{a} = \frac{\dot{n}}{2n} = -\frac{\dot{T}}{2T}$$

for secular changes in the semi-major axis a, period T and mean angular motion n of an orbit with $T \simeq 10^8$–10^9 yr. We use a fourth order Runge–Kutta[9] algorithm to solve the equations of motion in polar coordinates (r, θ) for a field $G \propto t^{-1}$. For all times after the epoch of unbinding, as judged from the total energy $E \equiv 0$, the radial expansion $r \simeq -r\dot{G}/G$. With Van Flandern's value for $\dot{G}/G$, this relationship gives an expansion velocity of 73 km s^{-1} Mpc^{-1} to components of erstwhile bound systems. This formulation and the value of $\dot{G}/G$ thus parallel the Hubble Law and the value of the Hubble constant, H. They may be identical.

When G varies with time it is necessary to consider the radial expansion component within all large scale associations of self-gravitating objects. Emphasis need no longer be placed on the usual limitation of the cosmological expansion, to the regions between groups and clusters of galaxies. The average mass of a galaxy is usually estimated from the internal motions of many objects or by application of the virial theorem to the velocities and separations found within rich clusters. These methods produce conflicting results[10], and typically $10 < \beta < 400$ where β is the ratio of the virial estimate to the mass identified by all other techniques. Expansion has a negligible effect on the dynamics of ordinary galaxies, which for $r < 30$ kpc have $r \lesssim 2$ km s^{-1}. The stability of groups and clusters can now be considered.

The orbits of 2 galaxies with a combined mass of $2.5 \times 10^{11} M_\odot$ were simulated using $G(t) = G_0\,(1.5 \times 10^{10})t^{-1}$, where G_0 is the present value, when initially circular orbits become unbound $r \simeq 2r\theta$ and

$$r = r_{\mathrm{lim}} \simeq 0.8\left[\frac{8MG}{5}\left(\frac{G}{\dot{G}}\right)^2\right]^{1/3}$$

Orbits with large eccentricity may be unbound at $\sim r_{\mathrm{lim}}/2$. The simulated orbits show that now, for comparatively large galaxies, $r_{\mathrm{lim}} \simeq 580$ kpc, in agreement with Holmberg's[11] observational result ($H = 55$ km s^{-1} Mpc^{-1}). Those initially circular orbits that now have a separation of ~ 1.5 Mpc simulate the properties of many small groups. These orbits have $r \approx 3r\theta$, so the observed r.m.s. velocity dispersion $\langle V^2\rangle_{\mathrm{obs}} \simeq 10\,\langle (r\theta)^2\rangle$, and the virial mass is overestimated by a factor of ten. Small groups still persist because such orbits exist and have been unbound for only 10^{10} yr, and because $\langle V^2\rangle_{\mathrm{obs}}$ is a function of the characteristic size, R, of the group.

Estimates of the virial mass of the small groups are very inexact because $\langle V^2\rangle_{\mathrm{corr}}$ is the difference of two large quantities and is very sensitive to inaccuracy in either, and because it depends on the precise value of $\dot{G}/G$ and on the implicit assumption of an isotropic distribution of circular orbits. If the line of sight expansion component is calculated from

$$V_{\mathrm{R}}^2 = \frac{1}{3}\left(\frac{\pi}{2} R\dot{G}G^{-1}\right)^2$$

18 of the 50 groups with data listed by Rood, Rothman and Turnrose[10] have negative velocity dispersions, and the remaining data show a residual dependence of R on V. Thus with the Sculptor Group of galaxies[12], which have well determined 21-cm velocities for six of the seven core 'members' and independent estimates for most masses

$$\langle V^2\rangle_{\mathrm{corr}} \simeq 107^2 - 79.5^2 = 71.6^2 \text{ and } R = 1.2\ \mathrm{Mpc}$$

using data weighted by masses that are calculated assuming a common distance of 3.0 Mpc. When the data are weighted in proportion to the apparent luminosities, however,

$$R = 1.6\ \mathrm{Mpc}$$

and

$$\langle V^2\rangle_{\mathrm{corr}} = 100^2 - 105^2$$

Before the virial theorem can be applied it is necessary to determine an independent distance to every member. Those members receding almost in the line of sight have the smallest apparent separations from the group centre and are likely to be both the faintest objects and to have the largest recession velocities and conversely for approaching objects. Besides explaining the effect noticed by Arp[13], this process significantly biases the estimates of R and V.

The masses usually quoted for the Coma cluster refer to the well studied region with $r \lesssim 6°$. Using data from Rood *et al.*[14], $R_e = 3.7$ Mpc, $\langle V^2\rangle_{\mathrm{corr}} = 861^2 - V_{\mathrm{R}}^2 = 817^2$ so the adjusted mass from Poveda's[15] method is $M = 9\langle V^2{}_{\odot\mathrm{corr}}\rangle R_e G^{-1} = 3.0 \times 10^{15} M_\odot$ with $\beta \simeq 4$, and $M/L \simeq 120$. But when the outlying 'members' at radii of 14.2° (32 Mpc) are included[22], the lower limit to the mass is $1.3 \times 10^{16} M_\odot$ with $\beta \simeq 16$. This limit is inappropriate when G varies. Thus three particular orbits that result now in a separation of 32 Mpc from an initial orbit with eccentricity e at an epoch of 1.5×10^9 yr require

$$M_{\mathrm{Coma}} \begin{array}{l} = 2.0 \times 10^{14} M_\odot \text{ (if } e = 0.9\text{, start pericentre)} \\ = 1.2 \times 10^{15} M_\odot\ (e = 0.0) \\ = 2.3 \times 10^{15} M_\odot\ (e = 0.9\text{, start apocentre)} \end{array}$$

These are all an order of magnitude smaller than the above limit, and there is clearly sufficient flexibility in the range of possible orbits, to ensure the existence of solutions with $\beta \simeq 1$.

An extension to systems with extended mass distributions is possible with models such as King's[16] models of elliptical galaxies, which relate the mass to a core radius r_c through

$$M = 9\mu r_c \sigma^2{}_V\,(4\pi G)^{-1}$$

Here μ is a constant determined by the value of r_{tidal}/r_c adopted and r_{tidal} is the limiting radius. For NGC3379, King finds $r_{\mathrm{tidal}} \simeq 160 r_c$ so that any increase in mass for a model of given velocity dispersion σ_V is associated with a large increase in r_{tidal}. The mass has an upper limit when $r_{\mathrm{tidal}} = r_{\mathrm{lim}}$. For a brightest cluster–elliptical such as NGC4486 (ref. 17), $\sigma_V = 550$ km s^{-1}, $r_{\mathrm{lim}} < 4.7$ Mpc and $M < 10^{14} M_\odot$. When allowance is made for the predominantly radial orbits to be expected in the outer regions, these limits reduce to $M < 4 \times 10^{13} M_\odot$ and $r_{\mathrm{lim}} < 1.6$ Mpc. More self-consistent models should reduce this to $\sim 10^{12} M_\odot$. The variability of G here explains in principle both the observed small range in the absolute magnitudes of the brightest cluster–elliptical and its independence of the size and richness of the cluster[18].

The hypothesis of a variable gravitational constant can completely solve the 'missing mass' problem, and promises to assist the study of the formation, structure and evolution of galaxies. Already it is evident that Lynden-Bell's[19] violent relaxation method will be greatly facilitated. Most galaxies probably form by the fragmentation of a protocluster gas cloud, following processes like those described by Reddish[20]. With a steadily weakening gravitational field, the subsequent breakup of most early clusters into small groups and isolated galaxies is to be expected, and predicts the quasi-homogeneous distribution of single galaxies found by Turner and Gott[21].

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The Madagascar controversy still lives

We feel that the title and conclusions of the report by P. J. Smith[1] of new palaeomagnetic data from Madagascar[2] does not reflect an objective assessment of the arguments for the palaeo-position of Madagascar. In particular your correspondent considers that any geological evidence against a northerly derivation must now be considered anomalous and that the new palaeomagnetic information provides incontrovertible evidence for the movement of Madagascar from the East African coast in post-Lower Jurassic times. We would suggest that the Madagascar issue, which is critical in defining the fit of eastern and western Gondwanaland[3], is far from settled and that the new 'modern' data must be examined and evaluated against all other evidence: geological, geophysical and geochemical.

There seems to be a misunderstanding of the fundamental evidence against a northerly derivation of this micro-continent. The argument does not depend on the matching of coastlines, marine incursions and so forth, as such arguments are rarely diagnostic. In this particular case, the presence of Jurassic marine sediments in the Knysna outline in South Africa[4] suggests the presence of a Jurassic coastline along most of the Indian Ocean coast so that matches can be made in various localities (although the coincidence of late Jurassic–early Cretaceous volcanics on previously similar latitudes may be significant). The fundamental geological argument hinges on the existence in Kenya–Tanzania of thick sediments on-shore, and for considerable distances off-shore, that were deposited from early Karroo times (Upper Carboniferous) onwards. These sediments have been shown in numerous drillings and have been traced for several 100 km into the Indian Ocean basin by seismic profiles[5–7]. There seems to be no possibility that these deposits could exist in their present position if this was occupied by a micro-continent that has since moved away. Subsidiary indications against such a northerly fit are also indicated by the seafloor anomalies of the Indian Ocean[8] which show Madagascar in its present position some 75 Myr ago, at a time when India, which is placed against Madagascar in most reconstructions, was already level with or slightly south of Madagascar.

Samples from seven of the nine sampled sites in the Isalo Group of Madagascar have been thoroughly and carefully studied[2] and these palaeomagnetic observations, on their face value, are statistically consistent with only a northerly derivation of Madagascar. In view of the incompatibility of the palaeomagnetic and geological data, it is necessary to assess the possible sources of error in the palaeomagnetic data which can arise in excess of the statistical estimates. The sediments involved are continental sandstones and mudstones which, in modern environments, undergo extensive oxidation and haematisation over periods of many hundreds of thousands of years[9,10]. The within-site scatter of this limited collection is reported as being small[2] and to have been acquired over some time. The magnetisation is

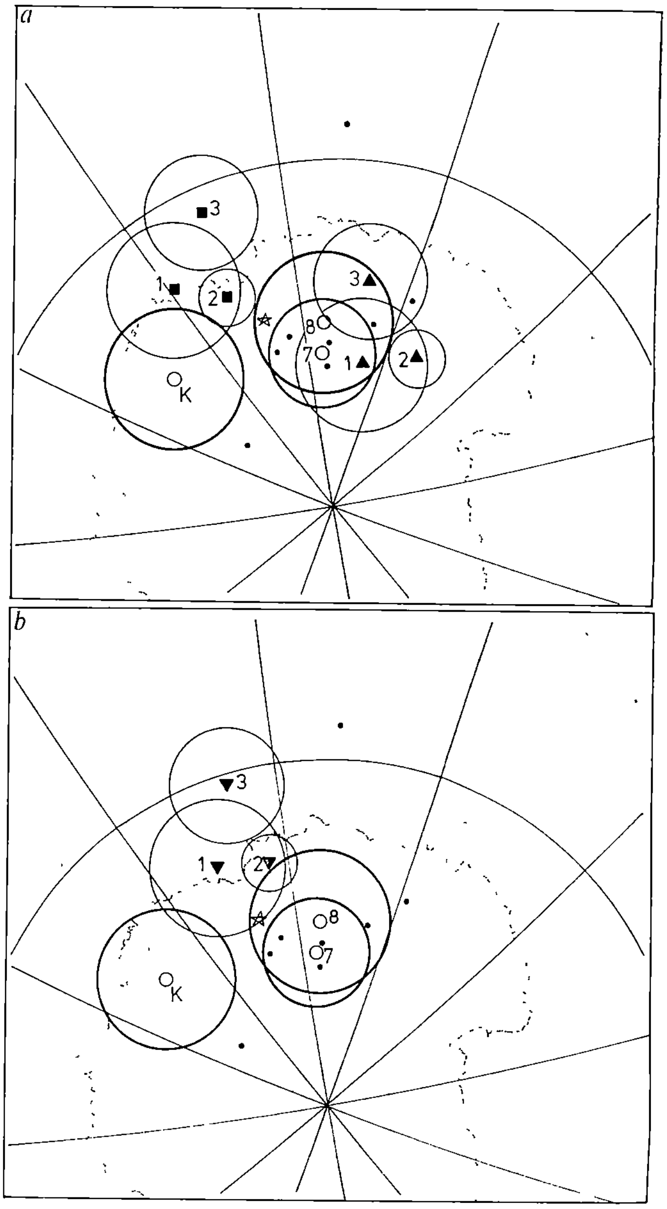

Fig. 1 Madagascan and African pole positions. Site poles from the Isalo Group (Triassic–Jurassic) of Madagascar are shown as dots, with the mean poles and associated circles of 95% confidence using all stable sites (8) and all well grouped sites (7) The mean pole position for Madagascan Cretaceous sites is also shown (K). The virtual geomagnetic pole corresponding to the present field direction in Madagascar is shown as a star. 1, 2 and 3 correspond respectively to the mean African Triassic–Jurassic pole used by Embleton and McElhinny[2], the Triassic–Jurassic site-mean pole from southern Africa and the Cretaceous site-mean pole for southern Africa. *a*, The Madagascan data are shown relative to the African data expected according to the Smith and Hallam reconstruction (▲) and the Flores position[16,17] (■) using the rotation suggested by Embleton and McElhinny[2]. *b*, The Madagascan data in relation to African poles (▼) assumed no differential movement between them during Mesozoic and Tertiary times. All circles are of 95% confidence.

not, therefore, of detrital nature, but is caused by chemical changes occurring after deposition. It is extremely difficult to isolate the magnetisation associated with detrital particles from that associated with later diagenetic changes[11], particularly as the detrital grains themselves may be changed during diagenesis and such haematisation of continental sediments may be delayed for at least 100 Myr, as in some Siluro–Devonian sediments[12–14]. It seems entirely possible, therefore, that the observed remanence is complex and that thermal and alternating magnetic field demagnetisation may not have effectively separated the different magnetic components, as has been suggested by Storetvedt[15] in connection with many continental sediments. It may be no coincidence that the pole corresponding to the present geomagnetic field in Madagascar (star on Fig. 1) is at least as consistent with the Isalo palaeomagnetic data as is the mean Triassic–Jurassic pole used by Embleton and McElhinny[2].

Even if the magnetisation is of similar age to the rocks, there are various ways in which comparison with other data of the same age can be made. Embleton and McElhinny[2] used data from all of Africa, assuming no differential movement between, for example, Sicily and South Africa, and also gave equal weight to each formation mean pole position. If only southern Africa data are considered, which are extensive and numerous, and the same weighting is used as in the analysis of the Madagascar data, that is, equal weighting to each site, then the result is significantly different from that of Embleton and McElhinny (Fig. 1). If anything, there is better agreement between the southern African pole (2) and the Madagascan pole (8) with both areas in their present positions in Triassic times. If the more scattered Isalo sites are omitted, then the resultant pole (7) differs almost equally from all three postulated positions. Smith[1] also ignores the Lower Cretaceous palaeomagnetic data from Madagascar[18–20] which are not consistent with postulated southern African Upper Cretaceous data, although furthest from the position expected if Madagascar was then in its northerly position or moving from it. We would not claim that our statistical analysis is superior or inferior to that used by Embleton and McElhinny, but the fact that different interpretations could be 'proved' by such analyses indicates that the quoted errors exceed the real errors involved in the comparisons.

The new palaeomagnetic data are therefore welcome additions to the available information relevant to the derivation of Madagascar, but there are clearly more grounds for considering the interpretations[1,2] of these as 'anomalous' than the vast range of geological and geophysical studies that are inconsistent with a northerly derivation. Nonetheless, we would prefer that the controversy remains open for further testing and consider that it is premature, and unscientific, to allow an uncritical acceptance of one line of evidence to suggest that further investigations are redundant.

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Grain-boundary migration in metals fatigued at high temperatures

THE migration of grain boundaries in polycrystalline metals fatigued at high homologous temperatures has been reported for FCC, BCC and CPH metals[1–4]. Migration results in a 'diamond' or orthogonal configuration of boundaries with segments aligned in the maximum shear stress directions. It has been suggested that this migration contributes to grain-boundary failure by the absorption of point defects at migrating boundaries[5] and/or by the development of the diamond configuration of boundaries, which puts these boundaries in a favourable arrangement for sliding[1–4]. In contrast to the information on cavity growth during high temperature deformation, there seems to be little quantitative information on grain-boundary migration in these conditions. We report here the results of measurements of grain-boundary migration on Al, Cu, Zr and Zircaloy-2 specimens fatigued at temperatures where grain-boundary cavitation occurs. These results indicate that in the initial 10% of the fatigue life the rate of boundary migration decreases with time and the amount of migration is $\propto \text{time}^{2/3}$. The possible influence of boundary migration on cavitation is suggested to be limited to the initial part of the fatigue life.

Table 1 gives the details of heat treatment, composition, grain size and test temperatures of specimens fabricated from sheet 1 mm thick. Specimens were chemically polished before testing in the reverse plane-bending mode of fatigue in high vacuum conditions (air pressure $<1.33\times10^{-4}$ Pa) at 16 Hz. The test conditions were typical of low strain–high cycle conditions, that is, the strain amplitude was in the range ±0.06–±0.257% which gave fatigue lives $>10^5$ cycles. At various stages of the fatigue life, the cyclic straining was stopped and the specimen allowed to cool to room temperature

Fig. 1 Log (average grain-boundary migration) against log (number of cycles) for: □, Al at 260 °C and a strain amplitude of ±0.073%; ◇, Cu at 500 °C and a strain amplitude of ±0.11%; ○, Zr (crystal bar) at 700 °C and a strain amplitude of ±0.125%; ×, Zr (crystal bar) at 700 °C and a strain amplitude of ±0.21%; ▽, Zircaloy-2 at 700 °C and a strain amplitude of ±0.21. The length of the vertical bars indicates the ± s.e. of the points. 'F' indicates failure.

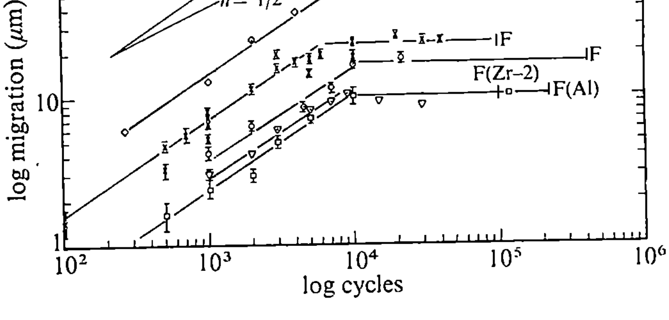

Table 1 Details of experimental technique

Metal	Test temp (°C)	Heat treatment (h at °C)	Purity	Grain size (μm)
Al	260	3 at 460	0.004 weight % total impurity	85
Cu	500	1 at 600	Commercial grade 0.014% O_2; 0.002% Mg; <0.05% As; <0.01% P, <0.01% Si	35
Zr	700	3 at 750	Crystal bar 0.00035 weight % O_2	60
Zircaloy-2	700	50 at 850	Standard commercial alloy 1.4% Sn; 0.14% Fe; 0.1% Cr; 0.05% Ni	60

under vacuum. The specimen was removed from the machine and examined under the microscope at a magnification of ×500 using a calibrated eyepiece. Grain-boundary migration was confined to the gauge length of the specimens.

In the case of Al, Zr and Zircaloy-2, the average migration distance, $\bar{m}$, was less than half the initial grain size and $\bar{m}$ was obtained from the widths of the migration traces left by at least 100 randomly selected grain boundaries[6]. For Cu, $\bar{m}$ was so large that traces left by migrating boundaries often covered whole grains, so that it was impossible to associate the traces with a particular boundary or a set of boundaries. In this case, $\bar{m}$ was estimated from $\bar{m}\simeq(\bar{D}-D_0)/2$ where D_0 is the initial grain size and $\bar{D}$ is the average grain size at a particular stage of fatigue testing. After repolishing the specimen, the grain size was measured using the linear intercept method.

The average rate of grain-boundary migration was found to decrease with increasing N and, after $\sim 10^4$ cycles or $\sim$10% of the fatigue life, approached a limiting rate close to zero. Values of $\bar{m}$ of up to 80 μm were obtained. Typical results are shown in Fig. 1 in which log $\bar{m}$ is plotted against log of the number of strain cycles, N. The initial part of the log $\bar{m}$ against log N curves for each of the materials can be approximated by straight lines of slope 2/3. The initial part of these curves can be represented by

$$\bar{m} \propto N^n \propto t^n$$

where n is a constant whose value is close to 2/3 and t is the test time. A least squares fit to 94 average values of $\bar{m}$ (representing over 9,400 individual measurements of boundary migration) during the initial stage of fatigue testing of Al, Cu, Zr and Zircaloy-2 gave a value of $n = 0.653$ with a s.d. ± 0.070.

Figure 1 also shows that the magnitude of $\bar{m}$ was dependent on both strain amplitude and material, whereas the initial slope, n, was independent of these factors. The value of n ($\simeq 2/3$) is greater than the upper limiting value of $n = 1/2$ for metals undergoing normal grain growth[7,8] or a value of $n = 0.31$ for acoustically assisted grain growth in Cu[9]. Comparison of this value of n with that for grain growth is, however, not strictly valid because of the different experimental conditions involved in each technique. The value of n obtained in our work for migration is in the range of the time exponents for cavity growth in Cu fatigued at high temperatures obtained by Gittins[5]. It should be noted, however, that the value of $n = 2/3$ for migration obtained in the present work applies only up to 10% of the fatigue life, whereas the exponent of 2/3 or 1 for cavity growth applies throughout the whole fatigue life. This suggests that in the present experiments, the absorption of point defects by migrating grain boundaries and their effect on cavity growth is only possible up to 10% of the fatigue life. This does not seem to have been considered in the migrating boundary model of cavity growth[5].

The suppression of grain-boundary migration at this stage of the fatigue test may be explained by three possible mechanisms:

(1) A balance may be struck in the defect concentration across boundaries[1]. This may occur when the cyclic hardening saturates.

(2) The rapid nucleation and growth of grain-boundary cavities during the first 60 s of fatigue testing, reported by Gittins[5], suggests that the presence of grain-boundary cavities would exert a significant drag on migrating boundaries[10]. The cavities may eventually grow to a size where they could prevent grain-boundary migration.

(3) The formation of the 'diamond' grain structure and the enhanced grain-boundary sliding that is possible for boundaries in this configuration may limit grain-boundary migration. At later stages of the fatigue life, where significant migration no longer occurs, the continued nucleation and growth of cavities would have to depend on other mechanisms such as vacancy capture or grain-boundary sliding. The applicability of these mechanisms is being examined.

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Extinction-free measurements in crystallography

OVER the past fifty or more years extinction has remained a major experimental problem in crystallography[1–3]. Many strategies have been devised to reduce its magnitude for the accurate determination of physically-significant structure factors (F). Its elimination, as a practical possibility, has appeared remote because extinction is a concomitant of the production of diffraction intensity in the standard techniques. Phenomenologically, extinction is a manifestation of multiple diffraction (see ref. 4), and this being so, an obvious conclusion is that the experimental disposition to be sought is one where the recorded intensity must arise solely from single-scattering events[5–7]. Then, by definition, the diffracted beam is not 'extinguished' and simple kinematical theory is applicable. Starting with the design of a 'defocusing' X-ray monochromator[8], I have been investigating the intensity of asymmetric reflections from extended-face crystals over the regions of both positive and negative asymmetry, first with an abraded surface[9] and then with an optically flat one. The results from this latter study, with an extrapolation of the work of Hirsch and Ramachandran[5], show that it is feasible to establish values of integrated intensity which involve only single-scattering events.

If one considers an asymmetric crystal in the 'defocusing' mode[8] (Fig. 1*a*) the incident beam enters the surface at a shallow angle. When diffraction occurs, the diffracted beam emerges through the surface by the shortest path consistent with the Bragg condition, and this gives the minimal possibility for the diffracted beam to interact with another part of the crystal and thus of energy to be removed by successive diffraction. As one approaches the limit of tilt, $\alpha = -\theta$, the range of penetration below the surface and the possibility of subsequent diffraction events decreases still further. Eventually only single

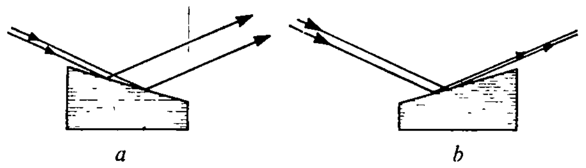


Fig. 1 Asymmetric reflection from an extended-face crystal (*a*) in the 'defocusing' mode with α negative and (*b*) in the 'concentrating' mode with α positive.

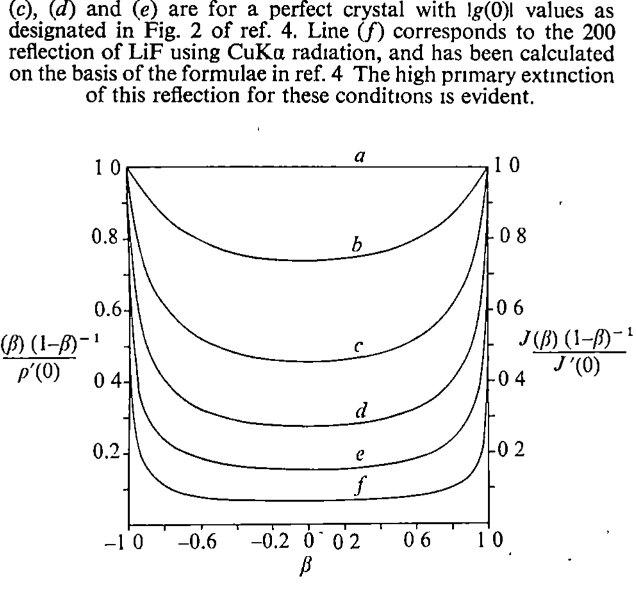
scattering events contribute to the emergent beam. The measured integrated intensity increases as $\alpha \rightarrow -\theta$, reaching a limit equal to twice the theoretical kinematical value at the symmetrical position $\alpha = 0°$, $\rho_0'(0)$ (refs 10 and 5). For the alternative 'condensing'[11] or 'concentrating'[7] mode (Fig. 1*b*), any diffraction processes beyond single scattering produce beams directed initially down into the crystal matrix. With increasing tilt, this trend becomes greater until, in the limit $\alpha = +\theta$, only single-scattering events contribute to the emergent beam. This disposition therefore acts as a filter to exclude multiple diffraction events. Near the limit, the emergent intensity tends to zero but its specific intensity tends to twice the corresponding kinematical value at the symmetrical position, $\alpha = 0°$ (ref. 5).

The significance of the optical finish of the surface for (1) the physical requirements of satisfactory extrapolations to $\alpha = \pm\theta$ and (2) ensuring minimum interference of counteractive absorptive effects from surface layers[9], including protuberances[6], is evident from the physical picture outlined above.

In terms of integrated intensity, the theoretical values range from the maximum of 2 at $\alpha = -\theta$ to 0 at $\alpha = +\theta$. If the measurements are normalised to effective equal scattering volume, as in Mathieson[9], the limiting values of $\rho(\beta)\ (1-\beta)^{-1}/\rho_0'(0)$ at both ends ($\beta = \pm 1$) of the range of the asymmetry β (defined as cot θ.tan α) are now unity. One may then recognise more readily the symmetrical relationship of the positive and negative tilt regions, in respect of both theoretical (Fig. 2) and experimental curves. In the latter case, the confirmation of symmetry is a valuable diagnostic.

The description above gives a physical interpretation of the theoretical curves presented by Hirsch and Ramachandran for the ratio of integrated intensity (and also specific intensity) of a perfect crystal with various levels of primary extinction, to the integrated intensity (and specific intensity) of an ideally-imperfect (mosaic) crystal of the same structure. These ratios, plotted against the asymmetry β, are shown in Fig. 2 of their paper[5]. Transformation of those curves for perfect crystals to a normalised basis is shown in Fig. 2 here.

Fig. 2 Transformation of Fig. 2 of Hirsch and Ramachandran[4] to show the functional dependence on the asymmetry factor β (defined as cot θ. tan α) of the normalised integrated intensity ratio, $\rho(\beta)\ (1-\beta)^{-1}/\rho_0'(0)$, and of the normalised specific intensity ratio, $J(\beta)\ (1+\beta)^{-1}/J_0'(0)$. The line (*a*) is for the ideal mosaic crystal (with zero secondary extinction). Lines (*b*), (*c*), (*d*) and (*e*) are for a perfect crystal with $|g(0)|$ values as designated in Fig. 2 of ref. 4. Line (*f*) corresponds to the 200 reflection of LiF using CuKα radiation, and has been calculated on the basis of the formulae in ref. 4 The high primary extinction of this reflection for these conditions is evident.



In ref. 5, however, the role of secondary extinction was not included in the theoretical model of the mosaic crystal. With secondary extinction included, the results for the mosaic crystal cannot be represented by the one straight line, (*a*) in Fig. 2. For real crystals there would be a family of curves dependent upon the magnitude of the secondary extinction, as in the case of primary extinction. The precise shape of curve would differ in detail from that for primary extinction but the relevant point is that the family of normalised secondary extinction curves, plotted as $\rho_s'(\beta)\ (1-\beta)^{-1}/\rho_0'(0)$ against β, would also converge to a value of unity at $\beta = \pm 1$.

For secondary as well as primary extinction, therefore, extrapolation of normalised values of integrated intensity to $\beta = -1$ and $\beta = +1$ yields numerical values of the integrated intensity which are extinction-free. Because they arise only from single scattering, the values can be equated to the simple kinematical formula and, in these circumstances, the necessary conversion factors, such as for the polarisation component, and the functional dependence on F^2, are well defined. Further, because the experiment involves the total incident beam, it is relatively straightforward to place the measurements on an absolute basis. To summarise—the real potential of the method is to establish absolute values of structure factors experimentally.

Note that Fig. 2 (with generalisations to include secondary extinction as noted above) clearly shows that, in regard to the reflected intensity, measurement in the symmetrical position, $\alpha = 0°$—the standard procedure with extended-face crystals since 1914 (ref. 12)—involves the maximum interaction associated with multiple diffraction and therefore the maximum extinction.

As a practical illustration of the procedure discussed here, the results for a 'strong reflection', the 200 of LiF with CuKα radiation, are presented. For this reflection, $\theta \simeq 22.5°$. A single-crystal boule was sawn at ~ 19.5° to the (200) plane, the surface ground and then given a finish flat to approximately one fringe in the working area. Measurements of intensity were made over the range $\alpha = -19.5°$ to $+19.5°$ (see ref. 8). After these were completed, a series of etches with HF were carried out, with intensities at selected tilt angles being measured after each etch. The intensity magnitudes dropped steadily and etching was continued until the values effectively stabilised. The state of the crystal surface was thus confirmed to be associated with secondary extinction. By contrast, the minimum value of intensity (measured at $\alpha = 0°$), attained after etching was completed and thus relevant to the interior of the crystal, corresponded to high primary extinction, that is, the crystal as supplied was near 'perfect'. In support of this contention, the base value for the matrix is shown as the solid circle in Fig. 3; the value lies close to the theoretical value at the symmetrical position of curve (1) which was calculated for F(200) of LiF and CuKα radiation for a 'perfect' crystal with the attenuation coefficient, $\mu = 32.2$ cm^{-1}, using the formulae in Hirsch and Ramachandran[5]. Line (2) corresponds to the theoretical mosaic crystal with zero secondary extinction. Curve (3) corresponds to the experimental measurements on the optically-flat asymmetric LiF crystal. A simple function was found to fit the 10 experimental points from $\beta = 0$ to $|\beta| = 0.845$, and this function was extrapolated to $|\beta| = 1$. One may note that the simple mean of $\rho(+\beta)$ and $\rho(-\beta)$ can be used.

While the above results establish the basic principle of this method, it will be clear that there is considerable scope for the exploration of both its experimental and theoretical aspects, especially close to the limits $\alpha = \pm\theta$ where the refractive index may require careful consideration, and a full report will be presented elsewhere.

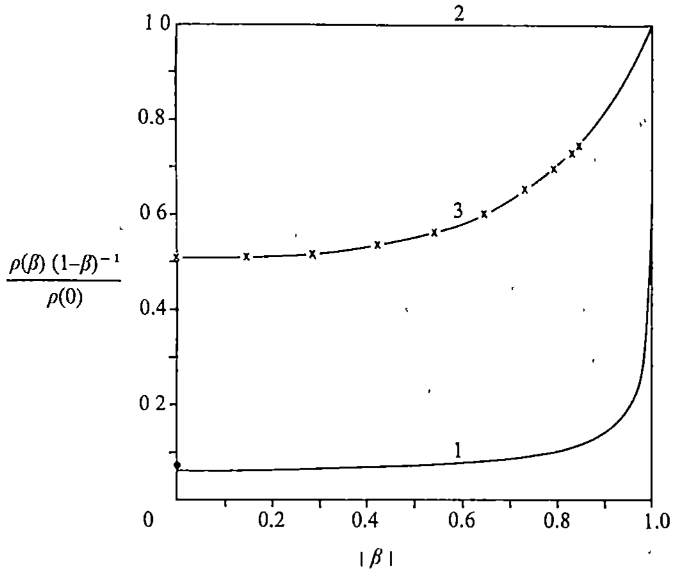


Fig. 3 Comparison of numerical data, experimental and theoretical, for the 200 reflection of LiF, scaled against the kinematical value as 1.0. The value, measured at the symmetrical position $\beta = 0$, for the single crystal after serial etching to reach the minimum intensity corresponding to the unstrained specimen, is indicated by the solid circle. Curve (1) is calculated for a perfect crystal of LiF, and corresponds to curve (f) in Fig. 2. Line (2) corresponds to the theoretical mosaic crystal with zero secondary extinction and is identical with line (a) in Fig. 2. Curve (3) shows the experimental points and their extrapolation to 1 0 at $\beta = 1$.

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Petroleum hydrocarbons inhibit decomposition of organic matter in seawater

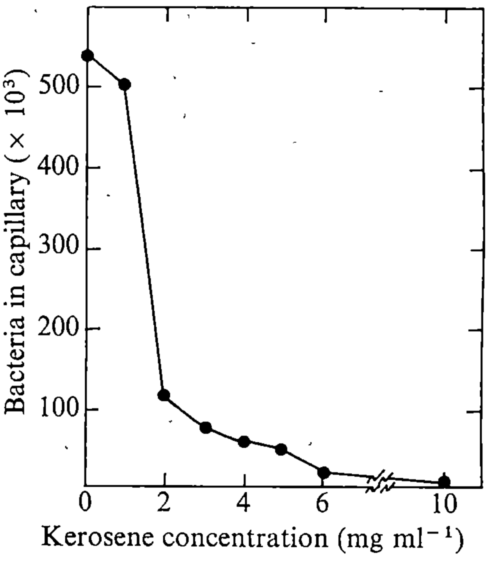
MOTILE marine bacteria, like higher animals, have the capacity to detect and be attracted to organic substances[1], including living microbial prey[2]. Mitchell *et al.*[3] found that this chemotactic response was inhibited by low concentrations of hydrocarbons such as toluene, phenol and crude oil. The bacterial population was not killed but did lose its ability to detect non-living substrates as well as living prey, such as algae and enteric bacteria[3–5]. It seems that the ability of a marine bacterium to degrade a substrate is augmented by its attraction to that substance. This report describes the role of bacterial chemotaxis in organic matter decomposition in seawater and its inhibition by sublethal concentrations of petroleum hydrocarbons.

Marine motile bacteria, which are capable both of utilising either albumin or casein as a sole carbon and nitrogen source and being attracted to them, were isolated from seawater using an enrichment technique[6]. Their chemotactic response towards soluble albumin or casein was tested using the method developed by Adler[7]. To test the ability of the bacteria to detect particles of organic matter in the seawater, the two test substrates were denatured by boiling at 100 °C for 10 min. The denatured products were only slightly soluble. Samples of 0.3 g albumin or casein were placed in nylon bags with 0.1-mm pores, permitting completely free contact of the bacteria in the water with the substrates. Bacterial suspensions (5 ml; 10^8 cells ml^{-1}) were placed at a distance of 12 cm from the substrate in natural seawater. The bacteria were attracted to the albumin or casein and utilised them as carbon and nitrogen sources. Enzymatic decomposition of the organic matter was determined both gravimetrically and by chemical analysis of protein using the method of Lowry *et al.*[8]. The role of chemotaxis in this process was estimated by blocking the chemoreceptor with the addition of petroleum hydrocarbons to the water. Kerosene was used as the source of petroleum hydrocarbons. Emulsification was achieved by sonification of the kerosene in the water before the addition of the bacteria and the organic matter. Figure 1 shows the effect of kerosene concentration on the number of bacteria attracted to the capillary containing an attractant. The results reveal that a concentration above 1.2 mg ml^{-1} kerosene significantly blocked the chemotactic activity of the bacteria. Motility was measured by monitoring the bacteria in a Petroff–Hauser counter under phase-contrast microscopy using a television camera and monitor to expand the scale. No inhibition of motility was observed within a range of kerosene concentration of 1.2–5.0 mg ml^{-1}. Above that concentration cell motility was adversely affected.

Figure 2 shows the effect of contact time on the blockage of chemoreceptors by kerosene. In this phase of the study we added 3.0 mg ml^{-1} kerosene to the seawater. The data indicate that within 10 min almost maximal blockage is achieved. Neither decrease in motility nor increase in chemoreceptor blockage was observed after this time.

Table 1 reveals a significant inhibition of decomposition of both casein and albumin in seawater in the presence of 3 mg ml^{-1} kerosene. This inhibition was overcome when the water was fully mixed using a magnetic stirrer. These

Fig. 1 Effect of kerosene concentration in seawater on the chemotactic response of marine motile bacteria towards albumin. Attraction to seawater alone: 6,500 bacteria per µl.



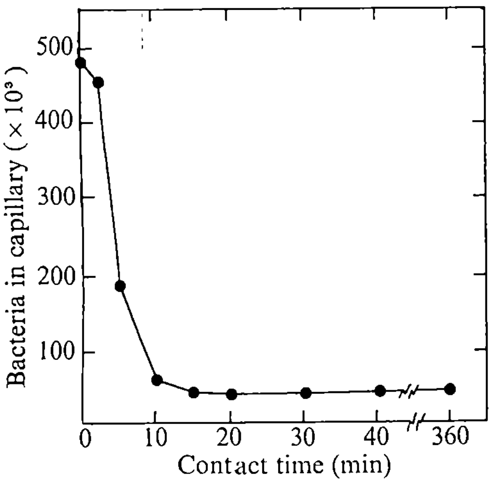


Fig. 2 Effect of contact time of marine bacteria with 3 mg ml^{-1} kerosene in seawater on the blockage of bacterial chemoreceptors Attraction to seawater alone: 6,500 bacteria per µl.

data show that, when chemotaxis was blocked, and the water was not mixed, no significant degradation of either albumin or casein could be detected. When random contact of the bacteria with the substrate was caused by physical mixing, however, 39% of the albumin and 65% of the casein were enzymatically degraded in 48 h, compared with almost 6% for albumin and 7% for casein in the standing cultures containing kerosene.

Table 1 Percentage of organic matter decomposition in the presence of 3 mg ml^{-1} kerosene after 48 h

Treatment	Albumin degradation (%)	Casein degradation (%)
Standing		
Sterile seawater	5.2	4.3
Seawater + bacteria	22.0	50.2
Seawater + bacteria + kerosene	5.8	7.4
Fully mixed		
Sterile seawater	8.8	10.0
Seawater + bacteria	30.5	48.4
Seawater + bacteria + kerosene	39.2	65.5

Our data show that chemotactic detection is important in bacterial degradation of these substrates; and that the kerosene concentration used in our tests blocks this function without inhibiting bacterial enzymatic activity. The relatively higher degradation rate in the physically mixed cultures containing kerosene can be partially explained by aeration and material loss resulting from the mechanical stirring (Table 1). The bacterial population increased by one order of magnitude from 10^6 ml^{-1} to 10^7 ml^{-1} in all of our tests. The phenomena which we observed do not seem to be the result of differential bacterial growth rates.

It seems that in seawater with a relatively low concentration of organic matter, bacterial chemotaxis may have a significant role in nutrient recycling. In polluted water chemotaxis inhibition may become a long term ecological problem since it can seriously undermine the capability of the marine microflora to decompose organic matter.

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Vulnerability of spatial frequency channels in cerebral lesions

Quantitative studies of vision in patients with cerebral lesions pose a challenge to purely topographical concepts of cerebral organisation. Pyschophysical measurements of the detectability of sinusoidal gratings (contrast sensitivity functions)[1] established that in the majority of patients detection of high spatial frequencies suffers most. The spatial frequency of a grating is the number of its alternating bars subtended in 1° of visual angle; the higher the number, the finer the pattern. There seems to be no obvious correlation between this loss of fine pattern discrimination and the aetiology or the exact localisation of a cerebral lesion. The neural basis of this loss is unknown in vision as with the other senses. Is it the result of specific vulnerability of those neurones of the primary visual pathways which are optimally responding to high spatial frequencies or to some defect of high order cognitive functions? In this report I present data on three patients who recovered from cerebral blindness. Serial evoked potentials and psychophysical detection of gratings of high frequency showed concurrent changes throughout recovery in one patient in whom they could be measured This suggests that structures of the central nervous system which determine evoked potentials and psychophysical responses to high frequency gratings are either identical or similarly vulnerable. These data also suggest why mid- and high-spatial frequency channels of the primary visual pathways may be especially vulnerable to cerebral lesions.

There is abundant evidence that gratings with sinusoidal luminance distribution may be optimal stimuli for cortical neurones of the mammalian[1–4] (including the human[5]) visual system. These stimuli approximate or match the spatial substructure of the receptive field of neurones of the primary cortex[6] and it has been suggested that neurones of similar spatial frequency optima constitute parallel "channels" of the visual system[7]. In the normal human psychophysical detection of gratings can be correlated with evoked potential measurements[5,8–10]. Studies of patients with cerebral lesions provide unique opportunities for investigating the effects of lesions on this correlation. In addition, evoked potential (EP) studies of patients can establish whether or not loss of fine grating detection occurs without damage to the primary visual pathways. If evoked potentials are intact whereas subjective detection suffers, then damage to higher order functions could be one possible explanation. If, however, evoked potentials and psychophysical functions change correspondingly, then it is not necessary to postulate damage beyond lesion of the primary visual pathways up to and including the primary visual cortex.

Over the years, 24 patients have been studied. In many of them evoked potentials and psychophysical measurements of grating detection are similarly affected by cerebral lesions[11]. In most patients clinical changes do not allow extensive psychophysical measurements, but in one rare case, serial evoked potential and psychophysical measurements were possible during recovery.

A 64-year-old woman, awoke one night with headache and inability to see, and was taken to hospital. Her neurological examination was normal except that she made errors in dates, although she was orientated to place. Her vision was almost limited to detection of light. Occasionally she detected large objects and gross motion. Pupillary responses were intact. Optokinetic nystagmus was present although irregular and asymmetrical when a regular inch tape stimulus was used and it was absent to a rotating drum stimulus. EEG, including alpha rhythm and brain scan were normal. Subsequent studies revealed that she suffered from cardiac arrhythmia with intermittent heart block and hypotension which most likely accounted for the sudden onset of cerebral blindness. Visual evoked potential measurements were obtained the morning after admission (Fig. 1). She was able to detect a 0.8 cycle degree^{-1} grating pattern when she was directed to look straight at the screen in front of her. This stimulus of 70% contrast produced evoked potentials in normal configuration. Evoked potentials were indistinguishable from noise when she detected no patterns above 2.1 cycles degree^{-1}. During her stay in hospital she progressively recovered peripheral vision and at the same time her spatial contrast sensitivity to foveally presented gratings improved as well. The recovery was apparent in the evoked potential measurements (Fig. 1*b*) and psychophysical and evoked potential measures of spatial contrast sensitivity were in agreement (Fig. 2). The noteworthy feature of these measurements was that evoked potentials provided evidence of damage of the visual pathways by confirming her subjective deficits. EPs were present in normal configurations when the patient said she saw the pattern and they were absent when she claimed no pattern detection. Furthermore, when evoked potential was present there was correlation (as in normals[8]) between EP amplitude and the contrast of the pattern.

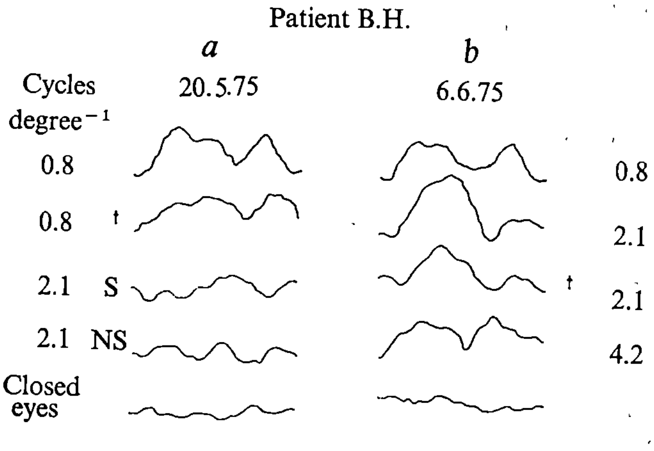

Fig. 1 Evoked potentials were recorded by silver disk electrodes placed on the scalp 2.5 cm above the inion and over the temporal bone corresponding to the posterior temporal electrode of the international 10-20 classification. The middle of the forehead was grounded. Potentials were filtered with corner frequencies of 1.5 and 60 Hz and averaged with a Nicolet 1062 computer, the active occipital electrode producing an upward deflection. Vertical gratings were generated according to standard methods[12,11] on the CRT of an oscilloscope screen at the mean luminance of 40 foot lamberts. The diameter of the screen subtended 4° at the eye. The stimulus grating was shifted to and fro at the rate of 8 Hz by a distance corresponding to the width of a single bar of the pattern called "counterphase flicker[13]. *a*, One day after the onset of cerebral blindness the patient detected a counterphase flickering grating of 0.8 cycle degree^{-1} of 70% contrast as well as 20% contrast († symbol). She occasionally detected a 2.1 cycles degree^{-1} grating of 70% contrast (S) to the left of the trace when she detected it; NS when she could not detect it. She could not detect any finer patterns that day. The duration of each trace is 125 ms and represents the average of 512 responses, or a recording time of 64 seconds. *b*, Marked improvement 17 d later. Evoked potentials were present to finer patterns than before, up to 10 cycles degree^{-1} (not shown) †, EP to a 2.1 cycles degree^{-1} counterphase grating of 20% contrast. Other traces obtained at 70% contrast. The number at the right is the spatial frequency of the pattern. The last trace was obtained when the patient closed her eyes. Calibration the same as (*a*).

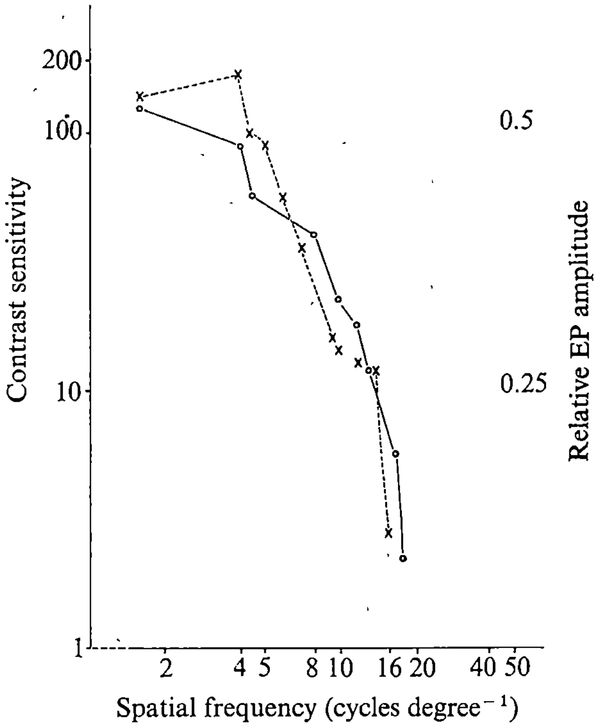

Fig. 2 Psychophysical and evoked potential measurements taken concurrently 5 weeks after admission. Psychophysical contrast sensitivity (○) and EP amplitude (×) are plotted as a function of spatial frequency. As our standard procedure[10], the method of constant stimuli was used to obtain frequency of detection curves. The psychophysical threshold is taken as the 50% detectable contrast. The inverse of contrast threshold is called contrast sensitivity. EP amplitude was measured as the peak-to-trough average of the quasi-sinusoidal waveform which is constant for 8 Hz counterphase stimulation, as shown in Fig. 1 for previously obtained data. Five weeks after admission there is still a considerable loss in contrast sensitivity compared with normal. This loss, expressed as a ratio of the normal to the patient's contrast sensitivity (not shown), increases with spatial frequency.

On admission to hospital the examination revealed that although the patient's peripheral vision and foveal fine pattern detection were absent some detection of crude patterns by foveal vision and occasional motion detection by peripheral vision remained. This suggests that there was damage to wide cortical areas, including peripheral "representation", but some foveal functions were intact. Potentials evoked by grating patterns recorded with the present method are largely determined by foveal contribution[8]; thus their presence soon after admission also suggests that some part of foveal "representation" was spared. The preservation of low but loss of high spatial frequency detection in the present case could be explained by the topography of lesions if detection of low and high frequency gratings by foveal vision were located in different areas of the calcarine cortex. Another possible explanation could be that representation of low spatial frequencies in parastriate regions received direct geniculate input (bypassing the primary cortex). Anatomical studies of the functional organisation of the visual cortex in primates do not provide support for these two possibilities[14–16]. Post-mortem studies in humans[17] show that grey more than white matter, the occipital pole more than the anterior part of the calcarine cortex, show damage after cardiac arrest, or after a short period of lowered blood pressure. Histological examination reveals laminar necrosis of layer IV (ref. 18).

As an alternative to topographical concepts another possibility must be considered. Single-cell studies[2–4,19] provide evidence that separate neurones with overlapping receptive fields respond to different grating patterns. This so-called spatial frequency selectivity characterises not only primate, but also human cortical organisation[5]. In this, as in other cases[1], recovery from cerebral lesions occurs *pari passu* with recovery of crude to finer pattern detection. This could occur if neurones optimally responding to low spatial frequencies were more numerous or had the greatest resistance to hypoxia or both. There is evidence that neurones of low spatial frequency optima belong to a functionally[19] different class than those with high spatial frequency optima but there is no evidence that there is a difference in numbers[8]. In addition to differences in function these two classes of neurones seem to have different neuropharmacological requirements[20,21].

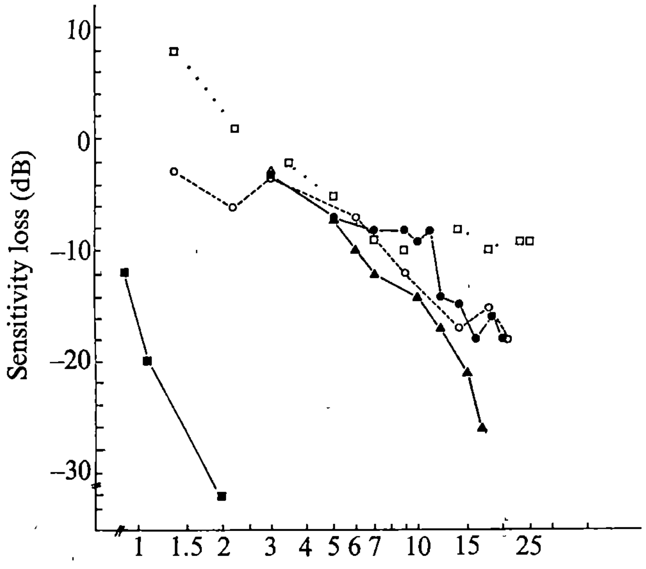

Fig. 3 Five visuograms demonstrate high frequency losses in three patients who recovered from cerebral blindness. A visuogram is the plot of the loss of the detectability of gratings, expressed in decibels as the ratio of the normal to a patient's contrast threshold at corresponding spatial frequencies. The gratings were presented in an on/off manner at the rate of 0.5 Hz. The degree of recovery of fine grating detection appears to be correlated with the time elapsed since the onset of blindness for an individual patient (dark symbols) and between patients. Solid symbols represent the data of the patient reported in detail on admission (■), two (▲) and four (●) weeks later. Empty circles show data of a 71-yr-old woman, and empty squares those of a 23-yr-old woman, respectively, 5 months and 5 yr after the onset of cerebral blindness.

This case and the two others summarised in Fig. 3 suggest that damage to neurones of the primary pathways which may be especially vulnerable to hypoxia, could explain high spatial frequency losses in humans. It remains to be seen whether or not metabolically vulnerable neurones of high spatial frequency optima can be identified in primates.

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Simulation *in vitro* of bovine host cycle of *Theileria parva*

Theileria parva is a tick-transmitted parasite causing East Coast fever in cattle. Research on *T. parva* has been impeded because of difficulties in completing the mammalian part of its life cycle in any non-bovine host. Several attempts to adapt the organism to small laboratory animals have failed[1–5]. Limited success was reported by Irvin *et al.*[6,7] using whole-body irradiated nude athymic mice. Many attempts to cultivate the parasite *in vitro* also failed[8–12] until Malmquist *et al.*[13] were successful using suspension cultures of infected bovine lymphoid cells which eventually contained the macroschizont form in the cytoplasm—the relationship between bovine lymphoid cell and parasite makes the perpetuation of both possible in a suitable culture medium. This led to further research into the development of an artificial immunogen and studies of the macroschizont stage of the life cycle without recourse to the bovine host. So far, however, tissue culture methods have not produced all stages of the parasite subsequent to the macroschizont; namely microschizonts, micromerozoites and piroplasms. The final stage, which is intraerythrocytic, is believed to be of crucia ilmportance in initiating the separate cycle which occurs in the tick vector (*Rhipicephalus appendiculatus*).

An infected bovine lymphoid cell line, established by the method of Malmquist *et al.*[13] from a prescapular lymph node biopsy, was used in the work reported here. Cultures were maintained in Eagle's MEM with Earle's salts, supplemented with 10% foetal calf serum (FCS) plus 0.1% extra l-asparagine. Experimental variations of this standard cuture medium showed that, by adding bovine lymph 5–10% and increasing the FCS to 20 or 30%, cells and parasites were stimulated to produce microschizonts, micromerozoites and, when bovine red cells were added to the cultures, piroplasms. The presence of the bovine lymph seemed to be associated with acceleration of mitosis of host cells with production of the later stages of the parasite as found in the bovine host. Mitosis of the cell was seen only when the parasite was in the macroschizont stage.

In one experiment is was shown that 50, 20 or 10% lymph added to cultures without FCS was toxic to the cells and an initial inoculum of 5×10^5 viable infected cells was completely non-viable by day 5. The addition of 20% FCS to medium containing 20 and 10% lymph seemed to provide the cells with sufficient nutrient to cope with the accelerated mitosis and parasite division with acceptable viable cell counts on day 5. The production of stages of the parasite other than macroschizonts was observed in smears stained with Giemsa. The utilisation of the nutrient material in the culture medium was emphasised in a further experiment. On day 3 half of each culture was withdrawn and added to fresh medium in a new culture flask. The viable cell counts of the original cultures continued to decline, whereas those provided with fresh medium showed at least a twofold increase after a further 2 d. Microschizonts have been observed at day 2 in such cultures and micromerozoites at day 3, except for one instance when they appeared at day 2. Ten experiments were set up in which washed bovine red cells were added to cultures of macroschizont-infected cells in medium containing bovine lymph. Microschizonts, micromerozoites and piroplasms were observed in 9 of these, of which 7 were 18-h cultures at the time of addition of the red cells, and the other two were 42- and 66-h cultures. The tenth culture was 90 h old before the red cells were added and there was no development of the parasite beyond the microschizont stage.

In smears made from the 9 cultures in which piroplasms were observed, only a small number of these were seen in each smear; estimated at 0.1% of red cells. Piroplasms were seen in these cultures in smears made over a period from 6 to 54 h after addition of red cells.

The intraerythrocytic location of the piroplasms was determined on the basis of shape and position of the parasites characteristic of those seen in the red cells of *T. parva*-infected cattle. In a few preparations an occasional micromerozoite was seen in an indentation of the envelope of a red cell, apparently in the process of entering the cell. Neither intraerythrocitic parasites nor parasites apparently in the process of entering the red cell were seen in any of the control cultures containing red cells and no lymph.

The above work shows that development of the parasite to its ultimate intraerythrocytic stage is possible *in vitro*. Experiments with further changes in the culture medium and/or conditions of culture may lead to a higher proportion of infected red cells in tissue culture, making the transfer of such infected material to the tick vector easier both *in vivo* and *in vitro* so that the whole life cycle of *T. parva* may be completed outside the bovine host.

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Cell-mediated cytotoxicity to SV40-specific tumour-associated antigens

CYTOTOXIC thymus-derived lymphoctyes (T cells) from mice infected with lymphocytic choriomeningitis virus, ectromelia virus, vaccinia virus, or parainfluenza virus interact only with H–2 compatible virus-infected target cells[1–6]. When congenic H–2 recombinant mice[7] and mice containing mutations within genes mapped at H–2K end specificities[6,8] are used, recognition of virus-infected target cells by the effector lymphocytes requires compatibility at either the *K* or *D* locus of the *H–2* gene complex. These observations led to the hypothesis that cytotoxic T cells recognise either a complex of viral and histocompatibility antigens or virus-induced alterations of the histocompatibility antigens. The same restriction has been described for the cytotoxic T cell response to lymphocytes modified by trinitrophenyl (TNP)[9], to minor histocompatibility antigens[10,11] and to the male Y antigen[12]. To investigate the possibility of a similarly restricted specificity of the effector cells of cell-mediated immunity to tumours, cytotoxic cells specific for SV40 tumour-associated specific antigens (SV40-TASA) were generated in two inbred strains of mice, C57BL/6J and BALB/cAn. The SV40-transformed lines C57SV (SV40-transformed C57BL/6 mouse embryo fibroblasts), MKS/Bu100 (SV40-transformed BALB/c kidney cells[13]) and LN-SV (SV40-transformed human skin fibroblasts[14]) were used as immunising and target cells (Table 1). In

Fig. 1 Time course of the generation of effector cells in mice immunised with SV40-transformed cells. C57BL/6J mice were immunised with a single intraperitoneal injection of 3 × 10^7 syngeneic SV40-transformed mouse–human somatic cell hybrid C121 (mouse parental C57BL/6 macrophages, *a*), allogeneic SV40-transformed hybrid C136 (mouse parental BALB/c macrophages, *b*) or xenogeneic SV40-transformed human cell LN-SV (*c*). The cytotoxic activity of spleen cells was tested on different days after immunisation. The target cells were: syngeneic C57SV (SV40-transformed C57BL/6 mouse embryo fibroblasts, ○), allogeneic MKS/Bu100 (SV40-transformed BALB/c mouse kidney cells, ●), and xenogeneic LN-SV (SV40-transformed human fibroblasts, □). Dose–response curves of specific cytotoxicity were determined by plotting ^{51}Cr-specific release compared with number of lymphocytes expressed in $\log_{10}$ units. The number of lymphocytes necessary to lyse 50% of 2 × 10^4 target cells in 12 h of incubation is referred to as 1 lytic unit.

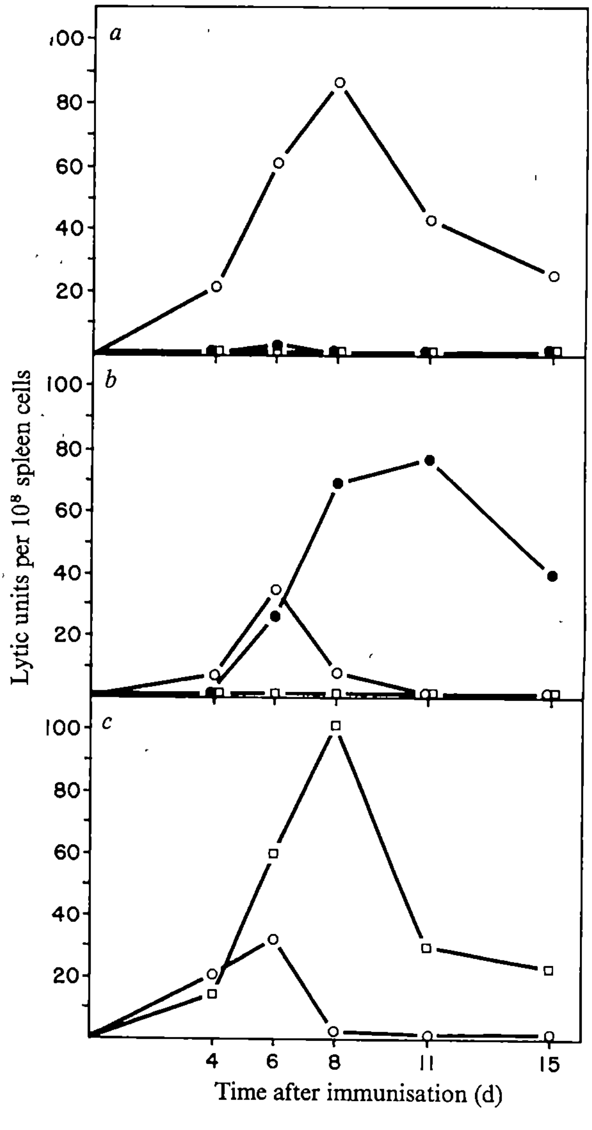

Table 1 *In vitro* cell-mediated cytotoxicity by spleen cells of mice immunised with syngeneic and xenogeneic SV40-transformed cell lines

			Responder strain	C57BL/6J	C57BL/6J	C57BL/6J	BALB/cAn
			Immunising cells	C57SV	C121	LN–SV	C136
			Time after immunisation (d)	8	8	6	8
			Incubation time (h)	12	12	18	20
Target cells	SV40 T antigen	Strain of origin	Human chromosomes				
C57SV	+	C57BL/6J	–	54	68	61	0
MC57G	–	C57BL/6J	–	0	3	4	0
C57BL EF	–	C57BL/6J	–	NT	NT	3	NT
C121	+	C57BL/6J	7	33	49	54	5
N9*	+	C57BL/6J	7	28	NT	57	NT
N8*	+	C57BL/6J	7	49	NT	60	NT
243B†	–	C57BL/6J	+	6	NT	NT	NT
MKS/Bu100	+	BALB/c	–	6	4	39	21.1
BALB EF	–	BALB/c	–	5	6	8	0
BALB/3T3	–	BALB/c	–	0	NT	5	2
C136	+	BALB/c	7	2	0	NT	37.8
NIH–SV3T3	+	Swiss random bred	–	NT	NT	25	NT
NIH–3T3	–	Swiss random bred	–	NT	NT	3	NT
LN–SV	+		All	8	0	59	2

C57BL/6J and BALB/cAn mice, 8–12 weeks old, were given a single intraperitoneal injection of 3×10^7 trypsinised cells. The effector cells were obtained from spleens of groups of at least three animals by gentle dissociation in a loose Potter homogeniser. The cytotoxic tests were performed in triplicate in the wells of flat-bottomed microtitre plates. Target cells were trypsinised and 2×10^4 cells and 2 μCi ^{51}Cr were added to each well. After 18 h the target cells were washed and effector cells in twofold falling dilutions were added in 200 λ medium. After suitable incubation times half the supernatant was removed for counting; the target cells were lysed with 1% Triton X-100 to measure the total releasable ^{51}Cr in each well. ^{51}Cr specific release, at a 100:1 ratio of effector to target cells was calculated according to the formula:

$$\%^{51}\text{Cr specific release} = \frac{(\%^{51}\text{Cr release in the presence of immune cells minus } \%^{51}\text{Cr release in the presence of non-immune cells})}{100 \text{ minus } \%^{51}\text{Cr release in the presence of non-immune cells}} \times 100$$

The standard error was usually less than 1%. The % ^{51}Cr release in the presence of non-immune cells was always either very close or identical to the release in medium alone, and ranged in the different target cells from 1.5 to 2.5% per h.

NT, Not tested.

* Clones of somatic cell hybrids obtained by fusion of LN-SV with C57BL or BALB/c peritoneal macrophages.

† Somatic cell hybrid obtained by fusion of an adeno-5 virus transformed human line[22] and C57BL/6 peritoneal macrophages.

addition, clones of human–mouse somatic cell hybrids, obtained by fusion of LN-SV and mouse peritoneal macrophages from inbred strains[15] were used. These hybrid clones contain the complete mouse genome and, in addition, one to several copies of human chromosome 7, in which the SV40 genome is presumably integrated[14,15]. C121, N8 and N9 cells are derived from LN-SV fused with C57BL/6 macrophages[16] and C136 cells from LN-SV fused with BALB/c macrophages[17]. These clones express the SV40 tumour (T) antigen (a nuclear antigen)[16,17], and the histocompatibility antigens of the murine parental cells (our unpublished results). Neither LN-SV nor any hybrid clones derived from LN-SV make infectious SV40[18]. Only after fusion with permissive monkey kidney cells can some SV40 particles be rescued. This defective virus exhibits no biological activity. Both LN-SV and human–mouse somatic cell hybrids containing the human chromosome 7 derived from LN-SV exhibit tumour-specific transplantation antigens (TSTA), as demonstrated by their ability to protect SV40-inoculated newborn hamsters from developing SV40 tumours[19]. Both the SV40-transformed mouse cell lines (our unpublished results) and the hybrid clones[16,17] are tumorigenic in immunodeficient "nude" mice, but these cells do not grow routinely in immunocompetent syngeneic mice, probably because of strong TSTA[20].

Mice were given a single intraperitoneal injection of 3×10^7 syngeneic transformed mouse cells or syngeneic mouse–human somatic cell hybrids. This led to the generation of effector cells in the spleen, with a peak of cytotoxic activity, as measured by ^{51}Cr release assay, 8 d after immunisation (Fig. 1*a* and Table 1). The cytotoxic cells were specific for syngeneic SV40-transformed cells. The cytotoxic activity was removed by treatment of the spleen cell preparations with anti-Thy 1,2 serum (Searle; AKR anti-C3H thymocyte sera) and complement. Allogeneic and xenogeneic cells, either transformed or not, were not affected by exposure to effector cells (Table 1). Cells from a syngeneic methylcholanthrene-induced tumour (MC57G), spontaneously transformed embryo fibroblasts (C57BL EF and BALB EF) and a hybrid clone from fusion of C57BL/6 macrophages with human adeno-5 virus-transformed human cells (expressing adeno-5 T antigen, F. K. Huebner, personal communication) were also not lysed (Table 1). The cytotoxic response generated in C57BL/6 mice was always several times higher than that observed in BALB/c mice; when BALB/c effector cells were used, a significant level of cytotoxicity, with restriction for SV40-transformed syngeneic cells, was observed only at longer incubation times (15–20 h, Table 1, column 4).

When the mouse–human somatic cell hybrids are used as immunogens they induce effector cells specific for SV40-transformed syngeneic mouse cells. When these hybrid cells are used as targets they are killed by cytotoxic cells induced by immunisation with syngeneic SV40-transformed mouse cells. Therefore these cells, which carry the SV40 genome integrated in the human chromosome 7, fully express SV40-TASA, which can interact with the mouse alloantigens to allow recognition by the effector cells. Additionally, immunisation of mice with these mouse–human hybrid cells does not result in generation of effector cells directed to surface antigens coded by human chromosome 7 since there is no cell-mediated cytolysis of human cells. These negative results contrast sharply with those obtained using sera of H–2-compatible mice hyperimmunised with the same human–mouse hybrid cells where significant antiserum activity to human cells is found[21]. The possibility remains that cytotoxic effector cells are generated specifically for the interaction between the human chromosome 7-coded antigens and the mouse alloantigens, but this cannot be demonstrated because of the simultaneous strong response to SV40-TASA on the hybrid target cells used.

The time course and peak of cytotoxic activity in the spleen of an animal immunised with SV40-transformed syngeneic cells (Fig. 1*a*) corresponds to that observed with xenogeneic antigens (Fig. 1*c*), and is slightly earlier than the peak of activity to alloantigens (Fig. 1*b*). After immunisation with SV40-transformed allogeneic (Fig. 1*b*) and xenogeneic cells (Fig 1*c*), any specificity for SV40-TASA on allogeneic or xenogeneic cells is masked by the simultaneous peak of cytotoxic activity against alloantigens and xenoantigens. The murine response to SV40-TASA, however, can be dissected in the allogeneic immunisation by the use of SV40-transformed syngeneic cells; that is when syngeneic mouse target cells are used, a peak of activity is seen 6 d after immunisation (Fig. 1*b*). After immunisation with SV40-transformed human cells, this cytotoxic response at day 6 is specific for SV40-transformed mouse cells (or mouse–human hybrids), regardless of their alloantigens (Fig. 1*c*, Table 1, column 3). This early cytotoxic response to syngeneic transformed cells after xenogeneic immunisation appears to be a T-cell response because it is (1) abrogated after complement-dependent lysis of effector cells with anti-Thy 1,2 sera, and (2) enhanced on a per cell basis after the effector cell population is eluted from a nylon wool column to remove adherent cells and significantly reduce the number of immunoglobulin-bearing cells.

The population of effector cells specific for SV40-TASA generated during immunisation with SV40-transformed syngeneic cells is apparently different from that generated by immunising with SV40-transformed allogeneic or xenogeneic cells. This hypothesis is based on two observations: (1) there is a different time course of cytotoxic activity against SV40-transformed cells after immunisation with allogeneic and xenogeneic cells (as compared with that after immunisation with syngeneic cells); and (2) there is a different specificity of the effector cells (which in the case of xenogeneic immunisation is not restricted to syngeneic target cells). LN-SV and its somatic cell hybrids cannot produce infectious SV40 in the mouse as the transforming virus is defective and mouse cells are not permissive for SV40 replication. Therefore, the immune response observed using allogeneic and syngeneic cells as targets is not due to the infection of host cells with subsequent expression of SV40-TASA. Moreover, any active infection of the immunised animal would result in the production of effector cells which could recognise only syngeneic target cells.

Our results indicate that functional interaction between alloantigens and new antigens on cell surfaces is necessary for the generation of effector T cells not only to viral antigens expressed during acute infection, but also to TASA expressed by transformed cells. Moreover, in the SV40 system, the degree of the cytotoxic response against tumour-associated antigens obtained with syngeneic immunising cells and target cells is comparable with that observed against allogeneic histocompatibility antigens. This syngeneic cell-TASA response is at least three times greater than that obtained by immunisation with allogeneic or xenogeneic SV40-transformed cells, using syngeneic SV40-transformed cells as targets.

Cytotoxic effector T cells may have a role in immunosurveillance against tumours. *In vivo* H-2-dependent restriction of the specificity of cytotoxic effector cells might be involved in the immune control of tumour growth, enhancing the cytotoxic response of the host. This augmentation of the immune response to tumour-associated antigens might be heightened in normal outbred populations due to the heterozygosity for histocompatibility antigens, each of which can interact with the newly expressed antigens on tumour cells, eliciting different clones of cytotoxic effector cells[22].

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Alteration in membrane glycoproteins after type-C virus infection of murine fibroblasts

NEW proteins and glycoproteins appear on cell membranes after infection of the cells by type-C viruses[1]. Some of these new proteins are virus coded and are positioned in the membrane away from the sites of virus budding[2]. GP70, the major murine glycoprotein has been identified serologically on the membranes of virus-shedding cells[3], transformed but non-virus-producing cells[4], and on some chemically induced sarcomas[5]. The internal core protein p30 has been identified as a common antigen on membranes of type-C virus-infected cells of many species[6]. It has been suggested that this protein, possibly a product of degraded virus, can bind nonspecifically to virus-shedding cells[5]. Non-virion virus-coded antigens have also been described. The best characterised tumour-specific surface antigen (TSSA) is that found on avian sarcoma cells[7]. It consists of a major fucose-containing glycoprotein of molecular weight 100,000 and possibly a minor glycoprotein of 32,000. These glycoproteins do not cross react antigenically with the avian virion glycoproteins. A similar antigen, the feline oncornavirus membrane antigen (FOCMA), has been described on virus-infected feline cells[8]. This antigen has not been well characterised but it is also thought not to be related to the virion proteins. Evidence has been sought largely unsuccessfully for a comparable TSSA in murine model systems[9]. There have, however, been a few reports of sarcoma specific antigens[10]. In this paper we describe alterations which occur in membrane glycoproteins after infection of murine fibroblasts by Moloney leukaemia–sarcoma virus (MSV–MLV-M) and by an N-tropic virus isolated from leukaemic AKR mice (MLV–AKR). The technique of lectin chromatography is used to fractionate glycoproteins from other membrane proteins enabling better characterisation of these important surface molecules.

Virus was isolated from tissue culture fluids by sucrose gradient centrifugation. ^{3}H-leucine-labelled cells were disrupted by nitrogen cavitation and the plasma membranes prepared by the two-phase method of Brunette and Till[11].

The virus and membrane samples were then dissociated with 1% deoxycholate (DOC) in water and glycoproteins were isolated and partially fractionated by affinity chromatography using *Lens culinaris* (LcH) lectin which is specific for α-D-mannopyranosyl-like residues[12] and *Ricinus communis* (RCA_I) lectin, which is specific for β-D-galactopyranosyl-like residues[13]. The agglutinins were bound to cyanogen bromide activated Sepharose 4B (ref. 12).

Figure 1 shows the fractionation of MSV–MLV-M virus on LcH and RCA_I affinity columns. The major viral glycoproteins of Moloney virus bind to both LcH and RCA_I lectin columns and are resolved into two components on SDS gels having apparent molecular weights of 74,000 and 79,000 (Fig. 1*i* and *j*). Figure 1*i*, *j*, *e–h* shows that the selective binding of glycoproteins to the LcH and RCA_I affinity columns separates the viral glycoproteins from proteins of similar electrophoretic mobility and apparent molecular weight which are presumably non-glycosylated. For example, proteins of approximately the same apparent molecular weight as the viral glycoproteins have been identified as precursors of the non-glycosylated viral group-specific antigens[15,16]. The ability of lentil lectin to discriminate between glycoproteins and non-glycosylated proteins was verified by using the periodic acid–Schiff (PAS) technique[20] to detect carbohydrate containing proteins on a sodium dodecyl sulphate (SDS) gel of lentil lectin fractionated Moloney virus (data not shown). Therefore, this simple procedure facilitates the isolation of viral glycoprotein. A glycoprotein of the MLV–AKR virus was isolated in a similar manner and found to be a single entity of molecular weight 79,000, which bound to both LcH and RCA_I lectin columns (data not shown).

Infection of secondary cultures of mouse embryo fibroblasts (Taylor's Own mice (TO), ICRF) with MSV–MLV-M results in the appearance of three new glycoprotein bands revealed by SDS gel analysis of the membrane preparations. Two of these glycoproteins bind equally well to RCA_I and LcH columns and have molecular weights of 74,000 and 79,000 (Fig. 2*d* and *f*). The lectin-binding specificities and molecular weights of these two glycoproteins are identical to the glycoproteins isolated from the purified virions. The third new glycoprotein which appears in infected cell membranes has an apparent molecular weight of 84,500 and is bound only by lentil lectin (Fig. 2*d* and *h*). A similar result was obtained after infection of TO secondary fibroblasts with a second preparation of MSV–MLV-M isolated from tissue culture supernatants from the MSC cell line[17].

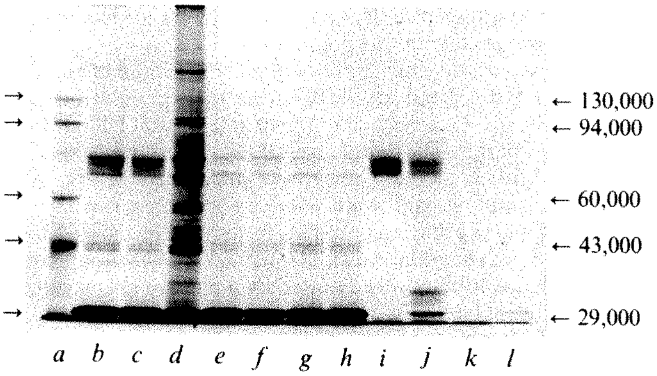

Fig. 1 8.0% SDS–polyacrylamide slab gel electropherogram (PAGE) of MSV–MLV-M fractionation on columns of LcH and RCA_1 lectin. MSV–MLV-M (5.0 mg) was disrupted in 12 ml 1% DOC (H_2O), sonicated for 30 s at 4 °C at position 4 on a Dawe Soniprobe (Type 7530 A) *b*, Centrifuged at 100,000*g* at 4 °C for 1 h (*c*, supernatant; *d*, pellet). A 5.0-ml sample of supernatant was applied to each of the lectin columns and washed through with 1% DOC (H_2O). The adsorbed glycoproteins were subsequently eluted with 0.3 M α-methyl-D-mannose (LcH) or 0.3 M D-galactose (RCA_1) in 1% DOC. The following samples were obtained: *i*, lentil bound; *j* ricin bound; *e*, lentil unbound; *f*, ricin unbound; *k*, ricin unbound → lentil bound; *l*, lentil unbound → ricin bound; *g*, ricin unbound → lentil unbound; *h*, lentil unbound → ricin unbound. Bands at the bottom of tracks *j* and *l* represent eluted ricin. Standards (track *a*) used for the purpose of calculating molecular weights were: β galactosidase (130,000); phosphorylase a (94,000); catalase (60,000); ovalbumin (43,000); and carbonic anhydrase (29,000). S.d. of molecular weight calculations ± 1,300. The gel system used was that of Studier[14]. The gel was stained with Coomassie blue.

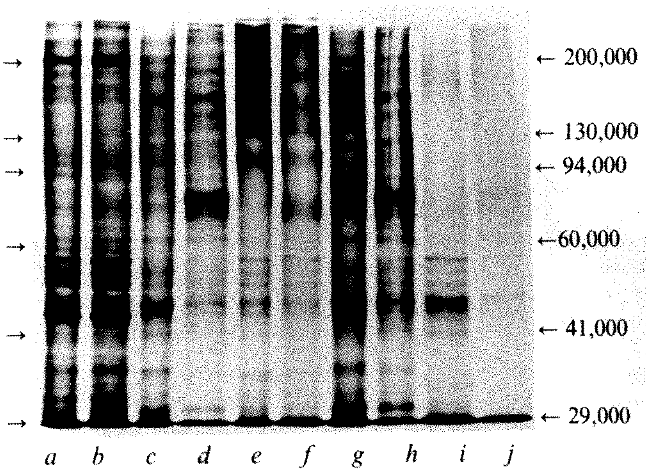

Fig. 2 Autoradiogram of 8% SDS–PAGE of membrane fractions of TO fibroblasts and TO fibroblasts infected with MSV–MLV-M (TO–M) after chromatography on columns of LcH and RCA_1 lectins. Cells were grown in Dulbecco's modified Eagle's medium with 10% foetal calf serum, and labelled for a 24-h period before collecting with ^{3}H-leucine (5.0 mCi; in 50 ml medium on 10 × 90 mm Petri dishes). Cells were collected by scraping, mixed with an excess of unlabelled SV40 B3T3 cells, washed with phosphate-buffered saline (PBS) and plasma membrane prepared by the two-phase polymer procedure of Brunette and Till[11]. The membranes were suspended in 1% DOC and the rest of the procedure was as in Fig. 1. 'Unbound' samples containing 150,000 c.p.m. and 'bound' samples containing 100,000 c.p.m. were applied to the SDS gel. After staining, the gels were impregnated with 2,5-diphenyloxazole (PPO)[18], exposed at −70 °C to Kodirex RP/R54 X-ray film for 5 d. Samples: *a*, TO; *b*, TO–M plasma membrane preparations; *c*, TO; *d*, TO–M lentil-bound fractions; *e*, TO; *f*, TO–M ricin-bound fractions; *g*, TO; *h*, TO–M ricin-unbound → lentil-bound fractions; *i*, TO; *j*, TO–M lentil-unbound → ricin-bound fractions. Standards (not shown) used are as described in Fig. 1 except that horse alcohol dehydrogenase (molecular weight 41,000) has been used in place of ovalbumin.

Comparative analysis of the membrane glycoproteins of the N3T3 cell line before and after infection by MLV–AKR (Fig. 3) reveals changes in a triplet of lentil-binding glycoproteins, ranging in molecular weight from 80,000 to 88,000 (Fig. 2*g* and *h*). These proteins have similar molecular weights to the new lentil-binding protein described in membrane preparations of MSV–MLV-M (TO–M) cells (Fig. 2). On infection of N3T3 cells with MLV–AKR the concentration of this glycoprotein triplet increases threefold. This increase was quantified by densitometry measurements. There is no alteration in intensity in any of the other membrane glycoproteins. Moreover, this triplet of glycoproteins binds exclusively to lentil lectin, whereas the viral glycoprotein binds both to lentil and ricin lectin columns (Fig. 3*f*). A direct comparison of the effects of infection by MLV–AKR and MSV–MLV-M in TO cells was not possible as TO cells restrict replication of MLV–AKR.

The lentil lectin-binding glycoproteins are most probably related to infection by MLV rather than to transformation by MSV as the effect was observed in cells infected by MSV–MLV-M in which the number of transformants was less than 1% of the infected cells. Moreover, the non-transforming MLV–AKR caused an alteration in similar lentil-bound glycoproteins.

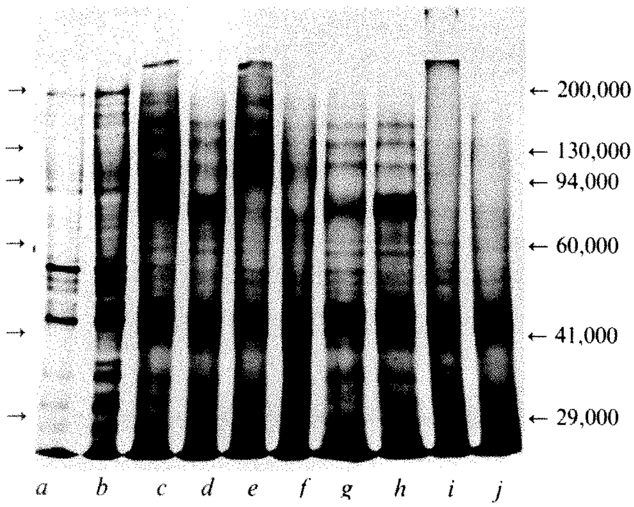

Fig. 3 Autoradiogram of 8% SDS-PAGE of membrane fractions of N3T3 cells and N3T3 cells infected with MLV-AKR after chromatography on columns of LcH and RCA$_1$ lectins. Samples were prepared as in Fig. 2. Samples: *a*, N3T3; *b*, N3T3-AKR plasma membrane preparations; *c*, N3T3; *d*, N3T3-AKR lentil-bound fractions; *e*, N3T3; *f*, N3T3-AKR ricin-bound fractions; *g*, N3T3; *h*, N3T3-AKR ricin-unbound → lentil-bound fractions; *i*, N3T3; *j*, N3T3-AKR lentil-unbound → ricin-bound samples.

This glycoprotein(s) may be a re-expressed embryonic antigen, a tumour-associated protein similar to the avian TSSA (ref. 7) or feline FOCMA (ref. 8) antigens, or it may be a precursor to the envelope glycoproteins similar to the precursor polypeptides which have been identified for the major non-glycosylated group-specific proteins of the virus[15,16].

Expression of endogenous virus and/or viral proteins in cell lines maintained in tissue culture is not an uncommon finding. It is possible that the expression of the lentil-bound 'triplet' of proteins seen in the N3T3 membrane sample in Fig. 3*c* is an example of such an event. After viral infection of the N3T3 cell line the concentration of this triplet of proteins seemed to increase threefold (Fig. 3*g* and *h*). This may be the result of infection by the endogenous AKR virus. It has also been suggested that infection by an exogenous oncogenic virus can stimulate derepression of endogenous virus[21] and that the target specificity of the cellular immune response directed against the resulting tumour will be endogenous viral protein[19].

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Genetic mimicry by hepatitis B virus

THE genetic hypothesis concerning the distribution of hepatitis B antigen (HB$_s$Ag, Australia antigen) carriers was proposed by Blumberg[1] and states that susceptibility to the chronic asymptomatic carrier state is determined by a simple autosomal recessive gene (Au^1). Persons homozygous for this gene (Au^1/Au^1) should become chronic carriers of the virus when infected with hepatitis B virus (HBV), whereas those heterozygous (Au^1/Au^0) or negative for the susceptibility gene (Au^0/Au^0) should not. Asymptomatic carriers of HB$_s$Ag are presumably chronically infected with HBV. Although independent segregation analyses of HB$_s$Ag in the families of blood donors[2,3] and natural populations[1,4-6] have failed to reject this hypothesis, it cannot account for the occurrence of a set of identical twins in which one twin had antigen, whereas the other had antibody[2]; nor for children with anti-HB$_s$ or without antigen in families where both parents were carriers of HB$_s$Ag (refs 3, 6 and 7). Indirect support for genetic control of the outcome of HBV infection comes from studies of thalassaemic patients in Italy[8] and asymptomatic carriers in the Solomon Islands[9]. These have suggested that susceptibility to the chronic HB$_s$Ag carrier state may be linked to heterozygosity of *Gm* genes and the resulting inability to synthesise as many kinds of anti-*Gm*. This hypothesis has not been supported, however, by subsequent investigations of other populations[10,11]. On the basis of results presented here of segregation analysis carried out on data from the Solomon Islands, we have been able to reject the existence of a susceptibility gene as a major factor leading to the HB$_s$Ag carrier state in this population.

We have carried out family and genetic studies of the distribution of HB$_s$Ag and anti-HB$_s$ in the total population of 771 individuals in Graciosa Bay on Santa Cruz Island in the Solomon Islands. The people were Melanesian, well nourished, and lived in leaf houses. They were apparently healthy at the time of sampling and were involved in normal village life. Reversed passive haemagglutination tests were carried out to detect HB$_s$Ag using the Auscell system (Abbott) with microtitre equipment. Antibody was detected by a passive haemagglutination test using lyophilised HB$_s$Ag-coated human red blood cells (Dr Lacy Overby, Abbott Laboratories). All positive reactions were confirmed by specific neutralisation by HB$_s$Ag, or anti-HB$_s$, whichever was appropriate.

Seventy-seven families were studied in which both parents were tested. Antigen was found in the children of 19 families. These were subjected to segregation analysis using the 'maximum likelihood' method and the method of Li and Mantel[12]. These analyses were carried out by Dr A. Barron, Department of Mathematics, Statistics and Computer Science of American University, Washington, DC.

Fifty-six children were investigated from 11 families in which both parents were negative for HB$_s$Ag and at least one child was positive. The maximum likelihood method was used to estimate θ, the probability that any given offspring would be a carrier of HB$_s$Ag (Table 1). We were testing the hypothesis that $\theta = 0.25$, which would be expected if the trait is an autosomal recessive. This analysis yielded a value of 0.182 ± 0.068. The comparison of this distribution with that expected for an autosomal recessive gave $\chi^2 = 0.901$. This indicates no signi-

Table 1 Maximum likelihood analysis of the distribution of HB_sAg carriers on Santa Cruz

Parental type	No. of children	θ	Expected	Standard deviation	χ^2
Negative × negative	56	0.182	0.25	0.068	0.901
Negative × positive	26	0.244	0.50	0.103	5.959

ficant difference between the actual and expected distributions. The method of Li and Mantel gave an estimated θ of 0.167 ± 0.081 (Table 2). This is within 2σ of the expected value of 0.25 and therefore supports the conclusion of the maximum likelihood method. From these data it is not possible to reject the hypothesis that $\theta = 0.25$; therefore the trait of being an HB_sAg carrier is distributed as an autosomal recessive.

In the group in which one parent was negative and one was positive for HB_sAg and at least one child was positive there were 26 children in 5 families. The genetic hypothesis would predict a value for θ of 0.50. The maximum likelihood analysis gave a θ of 0.244 ± 0.103 and a χ^2 of 5.959 (Table 1). This is significant at the 2.5% level and indicates that the actual distribution in these families is significantly different from that expected of an autosomal recessive trait. This is in contrast to the results obtained with families of negative parents. The method of Li and Mantel gave an estimated θ of 0.182 ± 0.123 (Table 2). This is not within 2σ of the expected 0.50 and therefore supports the conclusions of the maximum likelihood method. On the basis of this analysis the hypothesis that $\theta = 0.50$ and that the trait is distributed as an autosomal recessive can be rejected.

There were three families in which both parents were antigen carriers. These people have remained carriers of the antigen for at least 2 yr. The genetic hypothesis would predict that all children of these matings who were infected must become carriers. Of the 13 children in this group, however, only two were carriers, three had antibody and eight were negative for both antigen and antibody. The three children with antibody have certainly experienced infections with HBV but did not become carriers. This is clearly not compatible with the genetic hypothesis. Additional evidence against the genetic hypothesis is offered by a family in which both parents were negative for HB_sAg and all their four children were carriers. The probability of this occurrence according to the simple genetic hypothesis is 1 in 256.

A basic assumption in segregation analysis is that the genotype is reflected in the phenotype. We must assume that all healthy people who have HB_sAg in their serum are asymptomatic carriers and are homozygous for the susceptibility gene. We must also assume that we were able to detect all individuals susceptible to becoming carriers. These assumptions are probably not valid in the case of HB_sAg carriers for several reasons. First, although some asymptomatic persons may carry the antigen for many years[13], some may lose the antigen after a much shorter time[14]. Second, pregnant women with clinical hepatitis which does not develop into the chronic carrier state can transmit the infection to their infants who frequently develop chronic antigenaemia[15]. Presumably, these infants were not genetically predisposed to becoming carriers but rather became carriers because of their extreme immaturity at the time of infection. Finally, there are some people in all tested populations who show no evidence of having been infected with HBV.

Table 2 Li and Mantel analysis of the distribution of HB_sAg carriers on Santa Cruz

Parental type	No. of children	Estimated	Expected	Standard deviation
Negative × negative	56	0.167	0.25	0.081
Negative × positive	26	0.182	0.50	0.123

Given these reservations, however, our analysis of the children of matings between two carriers or between one carrier and one non-carrier rejects the genetic hypothesis, whereas in the same population the analysis of the children of two people who are not carriers of HB_sAg supports it. The importance of these data is not primarily related to the question of genetic predisposition to the HB_sAg carrier state because of serious objections to the validity of the application of segregation analysis to this type of data. This result, however, contradicts several other studies[1–6] using the same methods. It is possible that in other populations genetic factors may have a more important role, but in Santa Cruz there does not seem to be any simple autosomal recessive gene which significantly affects the distribution of HB_sAg carriers, although there is mimicry of genetic patterns by the offspring of normal parents. Data from Taiwan which were similar to ours were mentioned by Dr Beasley in a discussion at the National Academy of Sciences Hepatitis B Symposium[16]. In his study HB_sAg was found in 89% of the children of two positive parents, 55% when mother only was a carrier, 26% when father only was a carrier, and 23% when both parents were negative for HB_sAg.

A significant implication of this result lies in the possibility that chronic HBV infection may be representative of other infections of similar epidemiology with distributions that may also mimic genetic patterns. The observed distribution of carriers is probably the result of the prolonged presence of infectious carriers in combination with limited dissemination regulated by transmission routes involving exposure to infectious blood. Family clustering of antigen carriers may be the result of increased risk of exposure rather than genetically determined susceptibility[17]. It is possible that other diseases which are now accepted as genetically determined because of similar distribution patterns may also be caused by infectious agents. One area of particular interest would be that of polygenic birth defects which might be due to *in utero* infection with a virus from an asymptomatic carrier mother rather than the result of a complex set of interacting genes. In addition, late onset diseases which cluster in families may be due to infectious family interactions centred on an asymptomatic carrier rather than genetic predisposition.

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Drugs that disrupt microtubuli do not inhibit lymphocyte activation

LYMPHOCYTE activation by lectins is initiated by the binding of the lectin to a cell surface receptor. It is not known, however, how this event occurring on the cell membrane is converted into a signal that is transmitted to the cell interior. Edelman and coworkers[1–4] have advanced the hypothesis that lectin receptor sites may be attached to a submembranous microtubular system in a reversible way. This attachment could then determine the receptor mobility in the plane of the membrane and could also constitute a system for transmembrane signal transmission. The hypothesis is based, to a large extent, on the releasing effect that microtubule-disrupting drugs have on the inhibition of surface immunoglobulin cap formation in mouse B lymphocytes by concanavalin A (con A)[2,3]. Furthermore, evidence has been presented that colchicine delayed and suppressed the appearance of con A-induced blast cells in human lymphocyte cultures, that there is a delay in the onset of DNA synthesis in cultures containing colchicine and that drug sensitivity decreases with increasing culture time in the presence of con A[4]. Cap formation of surface immunoglobulin (Ig) is a phenomenon restricted to Ig-bearing B lymphocytes and these cells are not activated by con A. It is felt therefore that observations on this population do not bear directly on the relationship between the con A receptors and microtubuli as a signal-transducing mechanism. The other observations are suggestive but not conclusive for such a role of the microtubular system.

We have therefore performed experiments to test the hypothesis. If (1) a lymphocyte must have an intact microtubular system to be activated by con A; (2) the drugs disrupt the microtubuli in a reversible way, so that removal results in reassembly; and (3) if lymphocytes are preprogrammed, so that the timing of the sequence of events in a con A-activated cell is fixed, then it must be expected that the presence of microtubule-disrupting agents during the first 24 h of culture only, would result in a delay of the onset and peak of DNA synthesis. It would also be expected that an increase in RNA synthesis induced by con A would be inhibited in the presence of the drugs, since it is compulsory that this early RNA synthesis takes place before DNA synthesis and mitosis[5,6].

Table 1 Effect of microtubule-disrupting drugs on rat lymphocyte stimulation

	Thymidine incorporation (48–70 h) as % of control without drug		
Colchicine present in culture	Colchicine concentration (µM)		
	0.1	0.5	1.0
0–24 h	109	35	25
24–70 h	6	16	14
48–70 h	42	27	21
Vinblastine present in culture	Vinblastine concentration (µM)		
	0.1	0.5	1.0
0–24 h	120	124	97
24–70 h	120	105	92
48–70 h	120	105	99
Podophyllotoxin present in culture	Podophyllotoxin concentration (µM)		
	0.5	1.0	3.0
0–24 h	101	142	156
48–70 h	95	118	ND*
0–70 h	75	50	58

* ND, not done.

Table 2 Effect of microtubule-disrupting drugs on early RNA synthesis in con A-activated lymphocytes

Addition		Uridine incorporation (% of control)
Colchicine	0.1 µM	96
Colchicine	1.0 µM	99
Vinblastine	0.1 µM	95
Vinblastine	1.0 µM	150
Podophyllotoxin	1 µM	90
Podophyllotoxin	10 µM	117

Rat lymph node cells (2×10^6 per ml) were cultured during 24 h with 5 µg ml^{-1} con A. At 24 h, 2 µCi ^{3}H-uridine were added; 4 h later cells were collected and processed for counting. Uridine incorporation is expressed as percentage of control

$$\frac{\text{Increase in uridine incorporation with drug}}{\text{Increase in uridine incorporation without drug}} \times 100$$

Experiments were performed with lymph node cells from WAG/Rij rats of 10–14 weeks of age. Cells were cultured at 2×10^6 per ml in Hanks–Eagle's MEM supplied with 10% autologous fresh serum in tightly stoppered glass tubes.

Table 1 shows that with the exception of colchicine at 0.5 and 1.0 µM, none of the three drugs used inhibited thymidine uptake between 48 and 70 h of culture when they were present during the first 24 h of incubation. Colchicine was always strongly inhibitory when added later in the culture period and also when present during the labelling period only. This can be explained by the known inhibitory effect of colchicine on thymidine transport[7]. When colchicine was added at the same time as thymidine for a short labelling period (0.5–2 h), no inhibition of thymidine incorporation was observed. Apparently, the interaction of colchicine with the transport system is a sluggish process.

Vinblastine was not inhibitory at the three concentrations used; it even seemed to stimulate thymidine incorporation somewhat at 0.1 and 0.5 µM. Podophyllotoxin was found to

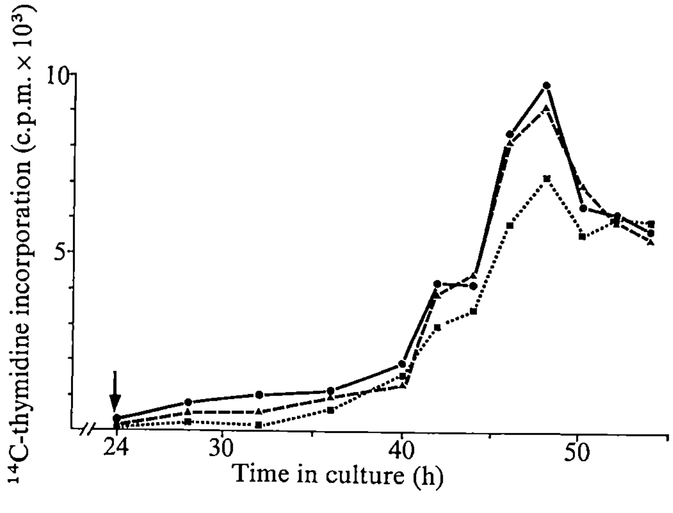

Fig. 1 Effect of podophyllotoxin on the time course of DNA synthesis in con A-stimulated lymphocytes. Rat lymph node cells (2×10^6 per ml) were incubated at 37 °C in Hanks-Eagle's MEM supplied with 10% autologous serum. Con A (5 µg ml^{-1}) and podophyllotoxin were added at $t = 0$. At 24 h the medium was changed (arrow) for one without podophyllotoxin. Cultures were labelled during 2 h with 0.15 µCi ^{14}C-thymidine (specific activity 50 mCi mmol^{-1}), collected by filtration on glass-fibre filters and the radioactivity counted. All points are the mean of triplicate cultures and data are expressed as counts per min in the presence of con A minus counts per min in the absence of con A. ●, No podophyllotoxin; ▲, 1 µM podophyllotoxin; ■, 5 µM podophyllotoxin.

Table 3 Effect of microtubule-disrupting drugs on rat lymphocytes stimulated by con A

Drug	% Blast cells at 48 h	% Blast cells at 72 h	Mitosis per 100 blasts	Viable cells (×10⁶) at 48 h	% Capping
None	37	77	3	1.53	5
Colchicine 1 μM	29	66	27	1.39	31
Vinblastine 1 μM	26	69	34	ND*	25
Podophyllotoxin 1 μM	37	77	26	1.62	16

*ND, not done

Blast cells and mitotic figures were counted in microscopic preparations made according to Sayk and stained with May–Grunwald Giemsa; 200 cells were counted. For the determination of the mitotic index, drugs were added at 40 h of culture and preparations were made 8 h later. Capping was determined as described by Yahara and Edelman[3] with FITC-conjugated goat anti-rat immunoglobulin; in the absence of con A, more than 95% of the fluorescent cells capped. Viability was determined according to Stewart and Ingram[8] with the Coulter counter.

be inhibitory only when present during the entire culture period. More accurate data on the onset and time of peak DNA synthesis were obtained from pulse label experiments. Cells were cultured during 24 h in the presence of the drug and con A. The medium was then removed and replaced by medium without drug. Cultures were subsequently pulse labelled at 2-hourly intervals and left exposed to the label for 2 h. The results of such an experiment with podophyllotoxin is shown in Fig. 1. The drug had no effect on the time of onset, the rate or the peak time of thymidine incorporation. Only the maximum incorporation was somewhat lower with the highest drug concentration. Essentially the same result was obtained with colchicine, except that the rate of increase of thymidine incorporation was slower than in the control during the first 12 h after removal of colchicine. This may be due to a slow release of the inhibition of thymidine transport into the cells.

Table 2 shows that the drugs had no inhibitory effect on the increase of uridine incorporation into RNA in the concentrations used. Table 3 shows that the drugs had the expected effect on surface immunoglobulin cap formation; they partly released the inhibition of anti-Ig induced capping imposed by a high concentration of con A. It also shows that all three drugs blocked mitosis in rat lymph node cells activated by con A. Both observations indicate that the agents can interact with microtubuli in the concentration range used in our system. Table 3 also shows that the drugs do not inhibit blast formation and are not cytotoxic.

Our results indicate that the three drugs in a concentration shown to disrupt the microtubular system do not delay con A-induced DNA synthesis, do not inhibit blast formation and do not inhibit lectin-induced RNA synthesis which precedes DNA synthesis. The results make it unlikely that an intact microtubular system is a strict requirement for signal transmission in lymphocyte activation by con A.

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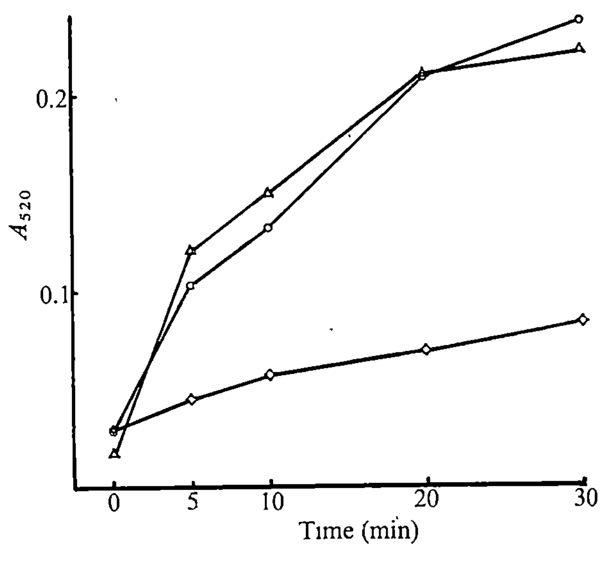
Synergistic effect of colchicine and cytochalasin D on phagocytosis by peritoneal macrophages

RECENTLY evidence has accumulated for the general involvement of microtubules and microfilaments in several membrane phenomena[1–8]. Usually, low concentrations of colchicine (μM concentrations) and related reagents have been used as the specific inhibitors for microtubules. In the case of phagocytosis, Ukena and Berlin reported that this reagent did not inhibit phagocytosis *per se* but affected molecular discrimination of membrane proteins during phagocytosis[2]. The mechanism of this interesting phenomenon has, however, not yet been explored.

On the other hand, higher concentrations of colchicine (mM concentrations) have been found to show an entirely different effect on several membrane phenomena. These include several-fold enhancement of Sendai virus-induced fusion of Ehrlich ascites tumour cells and of human

Fig. 1 Time course of ingestion of paraffin oil droplets in the presence and absence of colchicine. The system consisted of 1.7×10⁶ cells of mouse peritoneal macrophages suspended in 1.6 ml of Hanks' medium containing colchicine as indicated. After preincubation at 37 °C for 10 min with shaking, ingestion was started by the addition of 0.4 ml of prewarmed paraffin oil emulsion prepared as described in the text. Reaction was stopped by the addition of the medium containing *N*-ethylmaleimide and was assayed as described by Stossel *et al.*[12]. ○, No colchicine; △, 5 μM colchicine; □, 10 mM colchicine.

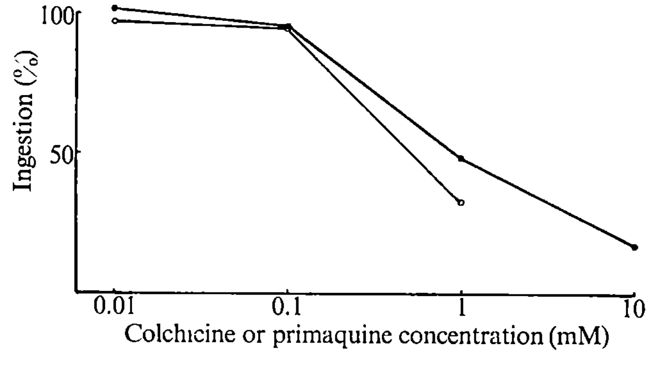


erythrocytes[9], and induction of energy-dependent internalisation of the membrane of human erythrocytes[10,11]. The purpose of this letter is to report the dual effect of colchicine on phagocytosis by mouse peritoneal macrophages and possible cooperation of contractile microfilaments and microtubules in this dynamic membrane reaction.

Phagocytosis of paraffin oil droplets was measured as described by Stossel *et al.*[12,13] except that Oil Red 5B was used instead of Oil Red O as a paraffin oil soluble dye. As Fig. 1 shows, colchicine had no effect on the rate of phagocytosis at 5 μM, but at higher concentrations it inhibited the reaction almost completely. The effect of different concentrations of colchicine on this process is summarised and depicted in Fig. 2. The colchicine concentrations required for inhibition of the reaction were similar to those used for stimulation of virus-induced cell fusion[9] and also for induction of membrane internalisation of human erythrocytes. Primaquine, the other inducer of membrane internalisation[10-11], was also effective as an inhibitor of phagocytosis at similar concentrations (Fig. 2). Inhibitory effect of these reagents therefore could not be associated with disruption of microtubules. Cytochalasin D, which interferes with microfilament function, is a potent inhibitor of phagocytosis at the same concentrations which inhibit actomyosin ATPase[14] and virus-induced cell fusion[9] (Fig. 3*a*). Effective concentrations of this reagent were found to be lower than those of cytochalasin B. Furthermore, the former is more specific than the latter: cytochalasin B both inhibits the function of microfilaments and facilitates diffusion of sugars through plasma membranes[15], whereas cytochalasin D is not inhibitory for membrane transport[9]. Accordingly, the participation of microfilaments in phagocytosis reported earlier on the basis of the inhibitory effects of cytochalasin B (ref. 16) is confirmed by the present experiments.

Furthermore, as depicted in Fig. 3*a*, low concentrations of colchicine were found to inhibit the reaction synergistically with suboptimal concentrations of cytochalasin D. A similar cooperative effect could be obtained with vinblastine instead of colchicine at concentrations which were reported to disrupt microtubules[17] (Fig. 3*b*). The effect of reagents which inhibit microtubules and microfilaments on phagocytosis is thus closely similar to those on capping of the surface immunoglobulin of lymphocytes[4-7]. This cooperative effect of inhibitors of microfilaments and microtubules may be explained by an increase in sensitivity of microfilaments component(s) to cytochalasins in the presence of microtubule inhibitors. Similar modulation of cytochalasin-sensitivity by extracellular Ca^{2+} has been found in the case of virus-induced cell fusion[18]. On the other hand, primaquine which behaved similarly with colchicine at millimolar concentrations did not mimic the effect of the latter at micromolar concentrations, in other words, no cooperativity was found between primaquine and cytochalasin D (Fig. 3*b*).

Fig. 2 Effects of colchicine or primaquine on the rate of ingestion of paraffin oil droplets. The system and conditions were similar to those described in Fig. 1 except that 3.8×10^6 cells were used and primaquine was employed instead of colchicine when indicated. Duration of the ingestion was 20 min. ●, Colchicine; ○, primaquine.



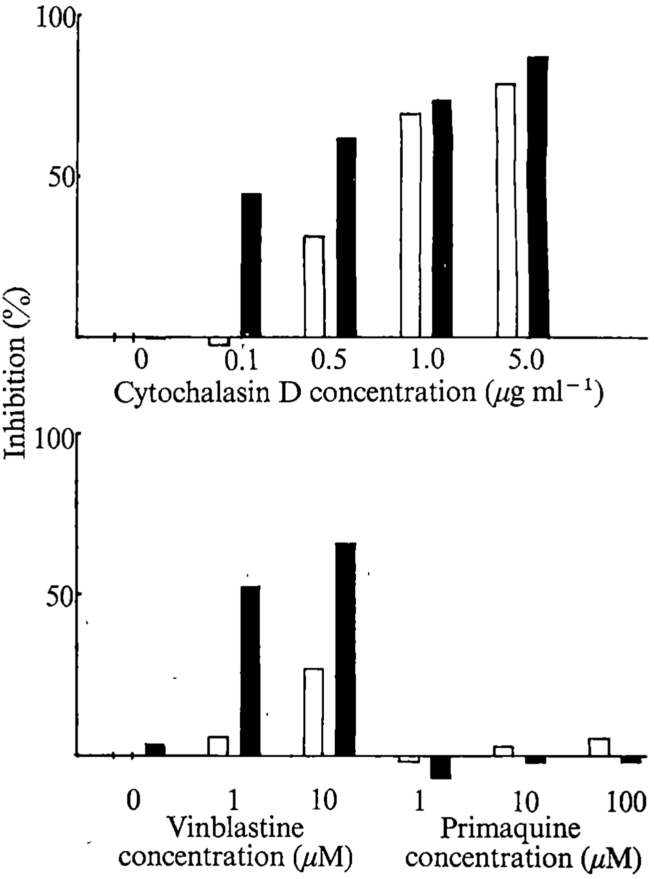


Fig. 3 Cooperative inhibition of phagocytosis with *a*, cytochalasin D and colchicine or *b*, cytochalasin D and vinblastine or primaquine. The system and conditions were similar to those described in Fig. 1 except that 3.6×10^6 and 9.5×10^6 cells were used in *a* and in *b*, respectively, and effectors were included in the system as indicated. Dimethylsulphoxide which was used for solvent of cytochalasin D was included in all tubes to a final concentration of 1% (v/v). Each bar represents the mean of 3–4 experiments. *a*, Open bar, cytochalasin D only; solid bar, cytochalasin D plus 10 μM colchicine; *b*, Open bar, either vinblastine or primaquine only; solid bar, 0.1 μg ml^{-1} of cytochalasin D plus either vinblastine or primaquine as indicated.

From the results described above, it could be concluded that these cytoskeleton components may name an important part in phagocytosis by mouse peritoneal macrophages. The possibility of cooperation of these components in cap formation has been reported before[4], and their cooperative participation in concanavalin A-induced agglutination of 3T3 cells has recently been reported[8]. Thus, membrane-cytoskeleton interaction seems to have some ubiquitous role in dynamic properties of biomembranes. Furthermore, it may be reasonable to assume that high concentrations of colchicine and/or primaquine influence phagocytosis by affecting some component(s) other than microtubules. Since similar concentrations of these reagents are known to affect the other membrane phenomena these effects of colchicine and primaquine are of considerable interest.

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Nuclear magnetic resonance studies of the adrenal gland and some other organs

KNOWLEDGE of the state of many chemicals in cell compartments is based on inference from analytical methods carried out after partial or complete disruption of the cell. This situation arises as most of the cell contents have similar spectroscopic properties and cannot be studied inside a cell. We have shown that the contents of intact vesicles of the adrenal medulla (chromaffin granules) can be studied by nuclear magnetic resonance (NMR) in separated vesicles[1]. (We have subsequently shown (unpublished observations) that the spectra are quantitative and that all the adrenaline present in the granules is observed by NMR spectroscopy.) From this study[1] we could establish an outline structure of the internal solution of these granules. The question then arises as to whether precisely this structure exists in the organelle within the cell or whether isolation of organelles such as these modified their properties. We report here that improved NMR methods can provide detailed information about the environment and mobility of small molecules and some proteins even within intact organs. As little as 10 mg (dry weight) of tissue is required. No other technique can provide such information at present. Furthermore, the NMR data can be used to describe structural features of the organisation of the cell.

The possibility of observing molecular species differentially by NMR spectroscopy arises from the following considerations. Nuclear magnetic resonance spectra of large aggregates of small molecules (for example, lipids in membranes) or of very large single molecules (for example, proteins of molecular weight greater than about 50,000) usually consist of broad, ill-defined absorptions over wide spectral regions, even at high magnetic fields. By way of contrast, highly mobile molecules (or parts of molecules) give rise to sharp proton resonances. Using convolution difference spectroscopy[2], we have shown that sharp lines can be separated from broad lines and we have used this method in the work described here. In addition, new methods of spectral simplification which depend on the use of T_1 and T_2 pulse sequences[3] allow separation of resonances which are undergoing different rates of nuclear magnetic relaxation, that is, sharp and broad resonances. In this way the narrow lines arising from rapidly tumbling molecules are separated from the broad resonances of the slowly tumbling molecules. It must be stressed that only resonances of highly mobile units are observed in the NMR spectrum in this work.

A typical NMR spectrum of the rat adrenal medulla from which the adherent inner cortex (the reticularis zone) has been removed is shown in Fig. 1*a*. This should be compared with the spectrum obtained from slices of bovine medulla (Fig. 1*b*). A similar spectrum is also obtained with slices of adrenal medulla from pig or sheep. Resonances which can be distinguished at 8.30, 7.95 and 5.89 p.p.m. are those of ATP H_8, H_2 and H_1, protons respectively while resonances at 6.78 (broad), 3.10 and 2.69 p.p.m. arise from the adrenaline aromatic, $-CH_2-$ and $-CH_3$ protons respectively. These

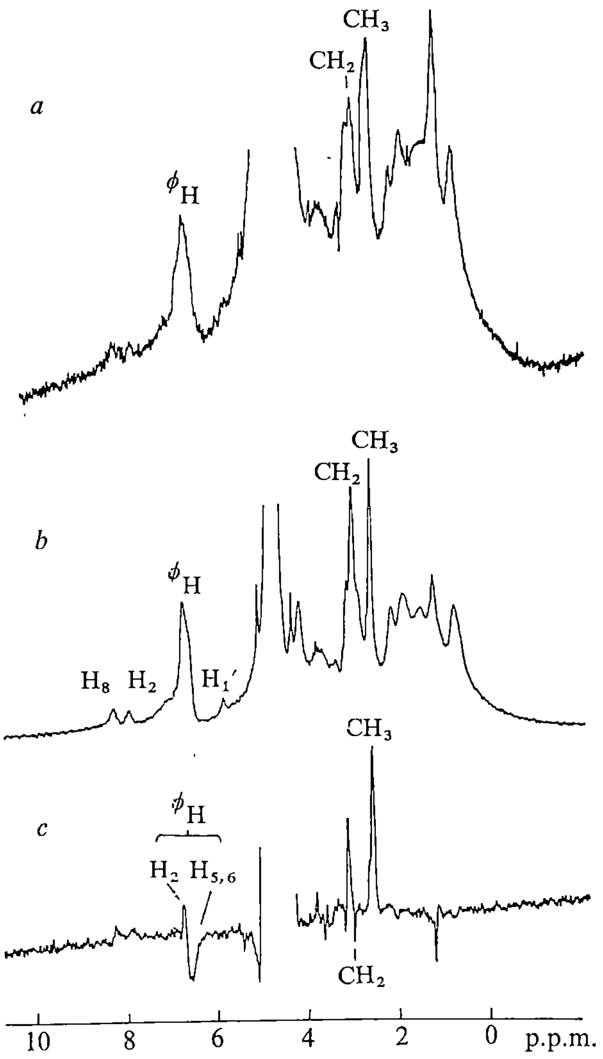

Fig. 1 Nuclear magnetic resonance spectra of dissected rat adrenal medulla, (*a*) bovine adrenal medulla slice (*b*) and bovine medulla slice (*c*) using a $(90°–\tau–180°–\tau–\text{echo})_n(T_2)$ pulse sequence. $\tau = 60$ ms. This pulse sequence separates resonances according to their respective transverse relaxation times, T_2. The component of magnetisation perpendicular to the applied magnetic field decays to zero during the delay time τ for resonances (of the macromolecule) with very short T_2 Consequently no peak (or only a very small peak) is observed for these resonances in the final spectrum. On the other hand, the resonances of the small, highly mobile molecule, adrenaline, are characterised by long T_2 and retain much of their original intensity when observed after the delay time τ Thus the adrenaline resonances are observed in the resulting spectrum while resonances of the macromolecular systems are reduced greatly in intensity or completely removed. The doublet resonances of the adrenaline $-CH_2-$ group and the aromatic H_5 and H_6 protons are inverted at the value of τ (60 ms) in the Fig. 1*c* due to modulation of the spin coupling[3]. Spectra were recorded at 19 °C for *a* and 23 °C for *b* and *c* using a Bruker 270 MHz spectrometer with an Oxford Instrument Company magnet. TSS was used as an internal standard. The adrenals were dissected from rats immediately after death and the cortex was separated carefully from the medulla by dissection under the microscope. The cortex and medulla were washed separately several times in a medium containing NaCl (160 mM), KCl (5 mM), $CaCl_2$ (2.2 mM) PIPES (2.5 mM), glucose (5 mM) and iproniazid (0.1 mM) in D_2O at *p*H 7.1. The spectrum of the isolated rat medulla (7 mg dry weight) was recorded using a Wilmad spherical NMR microcell assembly. Slices of bovine adrenal medulla were obtained from fresh adrenal glands after careful removal of the cortex and washed with the medium used for the rat medulla.

resonance positions are exactly those seen in the spectrum of isolated chromaffin granules[1], the peaks being shifted upfield with respect to the positions of resonances of the molecules in free solution. The resonances of adrenaline can be extremely effectively resolved by convolution difference spectroscopy or using $(180°–\tau–90°)_n$ (T_1) and $(90°–\tau–180°–\tau–\text{echo})_n$ (T_2) pulse sequences (Fig. 1*c*). When the slices are aged or incubated at about 50 °C, some of the adrenaline is released from the medulla and sharp resonances from free adrenaline $-CH_2$ and $-CH_3$ protons are observed at 3.21 and 2.77 p.p.m. respectively, with the aromatic nuclear resonances at 6.87, 6.93 and 6.95 p.p.m. These results confirm that the same adrenaline–ATP complex exists in the intact adrenal medulla as found in isolated chromaffin granules[1].

The peaks upfield from 2.5 p.p.m. in the spectrum of the beef medulla correspond to the protein chromogranin A, which is clearly in the same mobile, random coil state in the intact adrenal medulla as in the isolated vesicles[1]. The peak near 1.3 p.p.m. is, however, slightly more intense than is predicted for the amino acid composition of chromogranin, and this extra absorption probably reflects some small contribution from lipid fatty acid chain $-CH_2-$ groups. A shoulder near 3.2 p.p m is then likely to arise from the choline $N(CH_3)_3^+$ head group of a lecithin lipid. In spite of the very high lipid content of the adrenal medulla, no resonances of lipid protons are observed except for the two very weak signals mentioned above. Thus the medulla lipids must be in a state of slow molecular motion in which there is incomplete averaging of the dipolar interactions between spins resulting in severe broadening of the NMR resonances[4]. Further evidence for motional restraints of the lipid hydrocarbon chains is obtained from natural abundance ^{13}C NMR spectra of slices of medulla from pig or beef (our work in preparation) in which the chain methylene resonances are resolved only as a very broad featureless band. A sharper ^{13}C resonance arising from the choline $N(CH_3)_3^+$ group suggests greater mobility of at least some of the lipid headgroups. To investigate further the apparent rigidity of the membranes comprising the bovine adrenal medulla, ^1H NMR spectra were obtained for isolated chromaffin granule membrane, plasma membrane, mitochondrial membrane and microsomal membrane preparations. In each case a high resolution NMR spectrum was not observed although the resolution of a greatly broadened $N(CH_3)_3^+$ peak again suggested limited motions of the choline headgroup. Our results are in accord with those of previous NMR investigations of other biological membranes[4,5], which also demonstrated a state of slow molecular motion of the component lipids.

The ^1H NMR spectrum of rat adrenal cortex (predominantly the fasciculate and reticularis zones) obtained by dissection, under the microscope, of the rat adrenal gland after removal of the elastic capsule and adherent outer cortex (glomerulosa zone), is shown in Fig. 2*a*. The well resolved resonances are those of highly mobile lipid molecules. The observed lipid contains very little, if any, lecithin because no sharp choline $N(CH_3)_3^+$ proton resonance (usually the sharpest due to the high mobility of this group[5]) is observed. The high intensity of the CH=CH resonance at 5.33 p.p.m. and the relatively low intensity of the $-CH_2-$ peak at 1.30 p.p.m., show that highly unsaturated lipid is being observed. Such polyunsaturated fatty acids are known to comprise a sizeable proportion (about 30%) of rat adrenal cholesterol esters[6], and we conclude that the lipid signals in the NMR spectrum arise from lipid droplets, known to be abundant in the fasciculata (and glomerulosa) zone of the rat adrenal cortex[7]. (It should also be noted that, in intact plant seeds, we have obtained high resolution ^{13}C spectra (unpublished observations) from the lipid droplets which occur as cytoplasmic stores of fats and oils[8].) Although 5% of the wet weight of the rat adrenal cortex is cholesterol, about 75% of which is stored within the lipid droplets[9], no well resolved cholesterol resonances are observed in the NMR spectrum. When the convolution difference technique[2] is used, however, a weak signal is resolved at 0.70 p.p.m., which probably arises from the cholesterol 18-methyl group (Fig. 2*b*). This signal is too weak to account for all the cholesterol present in the liposomes—the remainder of the cholesterol must therefore be immobilised such that its NMR resonances are too broad to be observed. We note that cholesterol proton resonances are also absent from the NMR spectra of cholesterol–lipid systems[10].

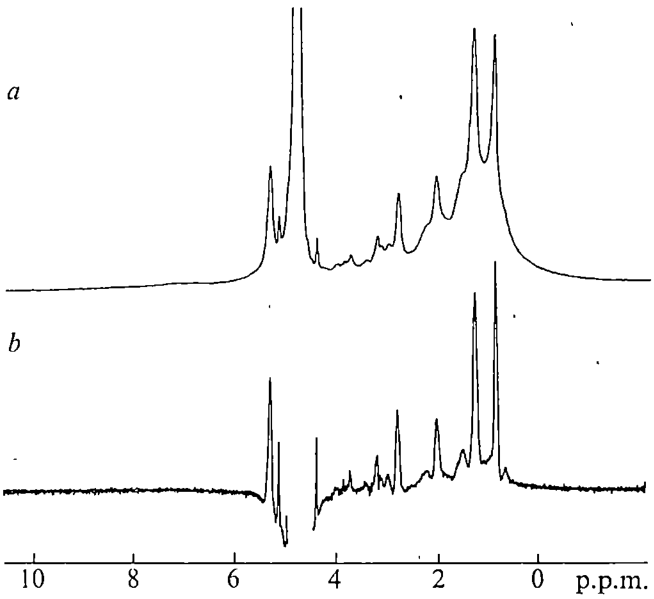

Fig. 2 NMR spectra of rat adrenal cortex (predominantly the fasciculata and reticularis zones). *a*, Normal FT spectrum; *b*, convolution difference spectrum showing a peak at 0.70 p.p.m. assigned to the 18-methyl group of cholesterol.

We now consider the advantages and new information which we have obtained from our ability to study the NMR spectra of whole organs. First, our observation of the NMR spectrum of the internal contents of the granules enables us to state that the dynamic structure of these granules, as far as ATP, adrenaline and the protein chromogranin are concerned, is the same in the whole organ as in isolated vesicles. Furthermore, this same structure is conserved in the adrenal medulla from four species. No other technique could lead to this conclusion. Second, evidence is obtained for the immobility of the lipid components of the adrenal medulla, in contrast to the state of rapid molecular motion of certain lipids in the rat cortex. Third, although the cortex is a storage organ for sterols much as the adrenal medulla is a store of adrenaline, the two types of store are in totally different physical states. Finally, the NMR technique is readily applicable to other intact biological tissues such as homogeneous cell cultures, and we have obtained well-resolved spectra from splenic nerve and blood platelets.

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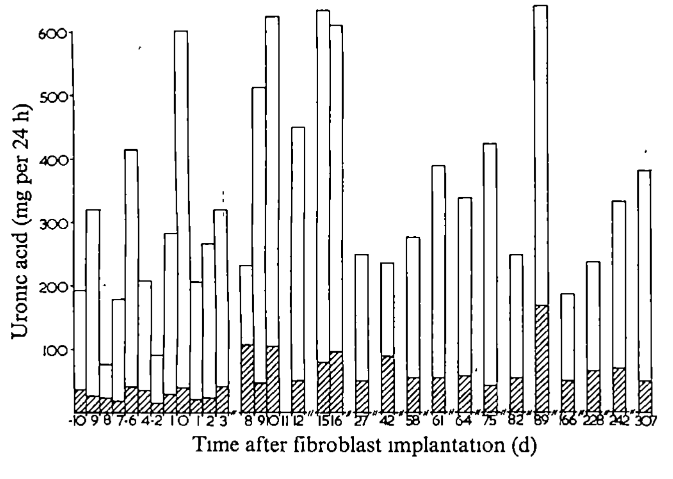
Enzyme replacement therapy by fibroblast transplantation in a case of Hunter syndrome

SUPPLEMENTATION of deficient enzymes essential for complete catabolism of glycosaminoglycans (GAG) has been used with limited success in several types of mucopolysaccharidosis[1–4]. The beneficial effects and concomitant changes in urinary GAG after this form of treatment, however, have been only transient, presumably because of the short life *in vivo* of the enzymes involved[5–7]. Because of this limitation, we recently tried, by means of skin transplantation, to provide a more permanent source of corrective enzymes in a patient with Hunter syndrome[8]. Although two HLA antigens from each donor were incompatible with those of the patient and both grafts had been visibly rejected within 3 months, there was a marked increase in breakdown and excretion of GAG subsequent to treatment, which lasted for more than 9 months. In addition, the activity of Hunter corrective enzyme isolated from the patient's urine, was also significantly increased. We attributed the effectiveness of the skin transplant to the release of Hunter corrective factor by donor cells and its uptake by host cells, in a manner analogous to that described for fibroblasts *in vitro*[9–11]. We have now attempted to increase further both the effectiveness and longevity of replacement therapy, using fully histocompatible skin fibroblasts injected subcutaneously as a source of corrective enzyme. An advantage of this procedure is that surgery is not required and it would in principle be applicable to other genetic deficiency diseases of lysosomal enzymes.

The patient chosen for this attempt was a 9-yr-old male (N.G.) in whom the diagnosis of Hunter syndrome was suspected on clinical features[12] and confirmed by correction studies of cultured fibroblasts using typed reference cells.

Fig. 1 Total 24-h excretion of urinary uronic acid before and after implantation of fibroblasts. Cross hatched areas of columns, polymeric uronic acid[13,14], open areas, oligosaccharides The uronic acid contents of all 24-h collections were calculated and the values depicted were selected as representative. Values for two age-matched normal subjects were: polymeric GAG per 24 h, 3.5 mg and 6.3 mg and oligosaccharide, 85.9 mg and 75.6 mg. The day on which fibroblasts were implanted is designated day 0 and the days before implantation by negative numbers.



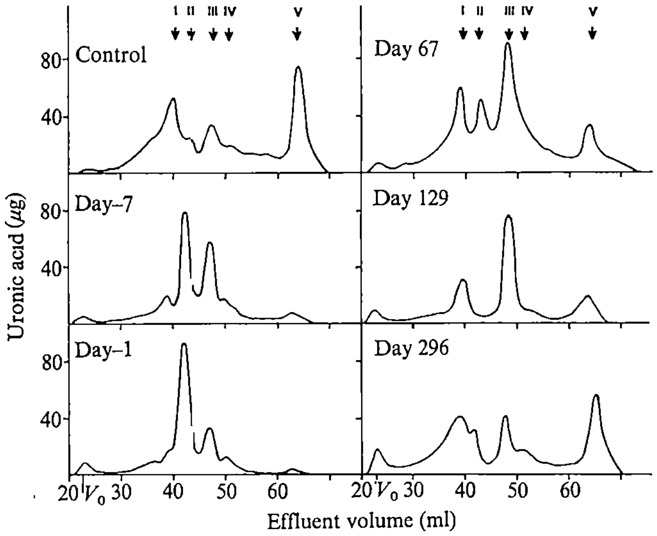


Fig. 2 BioGel P-2 filtration profiles of uronic acid oligosaccharides eluted from Dowex-1. Samples containing about 1 mg of uronic acid from each 24-h collection depicted in Fig 1 were chromatographed on a column (13 mm × 600 mm) of BioGel P-2, 200–400 mesh packed in and eluted with 0.2 M sodium acetate, *p*H 6.8 Fractions of 0.6 ml were collected. All samples were subjected to gel chromatography and the profiles shown were selected as representative of the changes observed and were unaffected on days of high (89) or lower (82) uronic acid output.

The patient and his sister, who was the donor, had identical HLA antigens, ABO and Rh blood groups (HLA: 2, 3, 12, W5; group O, Rh−ve). No reaction was detected when leukocytes from the patient and donor were mixed. The sister may have been a heterozygote (possibility 1 in 2).

The accelerated rate of incorporation of labelled sulphate by the patient's cultured fibroblasts before transplantation was corrected by culture of his cells in medium previously used to culture his sister's fibroblasts[10]. Normal donor fibroblasts were grown *in vitro* from a punch biopsy of skin taken from the sister. A suspension containing 2.2×10^8 cells was implanted subcutaneously into the patient in three dorsal sites. To minimise the risk of graft rejection, immunosuppressive therapy (Azothioprine, 25 mg daily and prednisolone, 50 mg daily) was begun 10 d before implantation, continued in the same dose for 6 months and then reduced progressively during a further 9 months.

Polymeric GAGs were precipitated[13,14] from aliquots of 24-h urine collections, taken from the patient before and after implantation and from a 10-yr-old normal female control The oligosaccharides remaining in the supernatant were collected on a column of 'Dowex' 1×2 (Cl^- form) washed with water, and then eluted with 2 M HCl and immediately freeze dried. Uronic acid contents were determined by an automated method[15]. Hunter corrective factor was isolated from the remaining volume of each urine collection and units of correction calculated as described before[8].

On the day fibroblasts were implanted the excretion of uronic acid increased to a level approximately double the preimplantation mean and to about 50% above the highest preimplantation value recorded. We attributed this immediate increase to Hunter corrective factor released by the fibroblasts into the medium in which they had been suspended before injection. The amount of uronic acid excreted per 24 h then fell and remained near preimplantation levels for several days, before increasing above pretreatment values (Fig. 1). This cyclical pattern was repeated several times during the period monitored but the overall

Table 1 Changes in percentage distribution of oligosaccharides eluted from a representative selection of BioGel P-2 chromatographs before and after implantation

Day of treatment	% Total uronic acid eluted in each component separated				
	I	II	III	IV	V
−7	13.5	41.6	29.0	12.2	3.7
−6	19.4	20.7	48.0	6.2	5.7
−1	15.7	55.1	17.2	10.0	2.0
+58	15.1	13.4	14.7	20.1	36.7
+89	16.9	5.3	14.5	43.2	20.2
+228	17.3	7.8	24.5	19.9	30.5
+296	33.8	8.0	15.4	7.6	35.2
Normal urine	36.7	6.2	14.8	9.9	32.2

Fractions I to V were numbered in order of decreasing hydrodynamic size as described in the text.

output of uronic acid was on average 50% greater than before implantation. Moreover, the polymeric component approximately doubled from the eighth day after implantation (Fig. 1).

When chromatographed on BioGel P-2 the oligosaccharides in normal urine separated into three major and two minor components (Fig. 2). The positions where the three principal components eluted in order of decreasing hydrodynamic size were: I, equivalent to the tetrasaccharides of chondroitin sulphate produced by the action of hyaluronidase (EC 3.2.1.35); II, equivalent to the monosulphated unsaturated disaccharide produced by the action on chondroitin-4-sulphate of Chondroitinase ABC (EC 4.2.2.4) (Miles); V, coincident with glucuronolactone. Although the oligosaccharides in the patient's urine were eluted at positions identical with those from normal urine (Fig. 2) before treatment, their relative proportions differed markedly (Table 1). Component II accounted for 20.7–55.1% of the total uronic acid eluted from the column, whereas in normal urine it represented only 6.2%. Likewise, the relative amount of component III was more than twice that in normal urine but the monosaccharide (component V) was less than 10% of that normally present. In addition a minor fraction comprising larger unresolved oligosaccharides was eluted with the void volume in samples prepared from Hunter urines.

After implantation, there was a progressive decrease in the relative proportions of components II and III (Table 1) and an increase in component V, until the overall elution profiles of the oligosaccharides were almost identical with those of normal urine (Fig. 2). The glucuronic/iduronic acid ratios of combined fractions II and III showed that more than 20% of the total uronic acid in these fractions was present as iduronic acid before treatment, whereas iduronic acid accounted for less than 10% some 2 months after implantation. At the same time the iduronate content of the monosaccharide fraction V increased (Table 2).

The activities of Hunter corrective factors isolated from the patient and from age-matched normal urines were calculated from their ability to correct abnormal incorporation of labelled sulphate by the patient's cells[8]. Four healthy boys aged 4–11 yr excreted 29.7–67.7±18.8 U (mean 45.1 U) of corrective factor per 24 h, whereas the patient excreted 0.0–1.04 U per 24 h (mean 0.47, $n=4$) before implantation. After implantation the excretion increased to 6.7% of normal values, with an average of 3.0±1.7 U per 24 h ($n=15$).

Table 2 Changes in relative proportions of iduronic acid present in oligosaccharides eluted from BioGel P-2 before and after fibroblast implantation

Day of treatment	Iduronate as % of total uronic acid	
	Fraction II+III	Fraction V
−7	27.8	19.8
−4	19.7	—
−1	21.7	17.6
+58	10.4	18.1
+65	8.0	27.5

Combined fractions II and III and fraction V were analysed by gas liquid chromatography as their trimethylsilyl derivatives after methanolysis[10]. Iduronic acid was calculated as a percentage of the total uronic acid detected.

In the severe form of Hunter syndrome, mental and physical deterioration progress relentlessly, with death often in the second decade. In our patient there was a marked improvement in general behaviour with loss of aggressive tendencies and no detectable deterioration in intellectual function during 10 months. His liver size diminished considerably but splenomegaly persisted. Full clinical assessment will be reported after a longer period of study.

Before replacement therapy can be effective, the level of deficient enzyme (sulpho-L-iduronate sulphatase) must be increased significantly and this increase must be sufficient to breakdown incompletely degraded heparan and dermatan sulphates stored within the tissues. All the changes that occurred after implantation, which were maintained many months afterwards, provide strong evidence that the corrective factor produced by the normal fibroblasts had enabled a significant proportion of the accumulated GAG and oligosaccharides within the tissues to be broken down to completion. Immunosuppressive drugs given before transplantation did not increase output of uronic acid and when gradually reduced the average excretion of uronic acid remained high.

The precise mechanism of action of the donor fibroblasts is as yet unknown, but it is assumed that they can release enough Hunter corrective enzyme to induce the observed effects when taken up by the host cells, as has been demonstrated for fibroblasts *in vitro*[9–11]. In Sanfilippo B syndrome a 2–5% increase in the intracellular level of α-*N*-acetylglucosaminidase can induce 42–70% correction in abnormal GAG metabolism *in vitro*[6]. If the same were true for Hunter corrective factor the increase in its activity observed after treatment might be sufficient to induce a large increase in catabolism of the accumulated GAG. Nevertheless, that so few normal cells should have an effect when transplanted is remarkable, but a small graft of skin in another Hunter patient[8] had a similar effect. It is possible that the active enzyme survives for longer than normal in the host. In the environment of the host, production of enzyme by normal cells may also be fully derepressed.

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Small molecular weight β_1 serum protein which specifically inhibits human collagenases

NEUTRAL collagenases are specific collagen-degrading enzymes which can be identified and isolated from the media of a wide variety of human tissues in culture. *In vivo* the enzymes function extracellularly at neutral *p*H and probably have a crucial role in the catabolism of collagen. Evidence for the involvement of the enzymes in diseases characterised by disordered collagen metabolism is substantial but our understanding of the mechanism of regulation of extracellular collagenase activity is poor. Since the specific inhibition of collagenase might offer a therapeutic approach to some connective tissue diseases we have attempted to identify the natural inhibitors of this enzyme in human serum. We report here our finding of a β_1 serum protein which specifically inhibits human collagenase. Because of its small molecular size (molecular weight about 40,000) this inhibitor may be important in the regulation of tissue collagenase activity.

It has been known for some time that whole serum can inhibit human collagenase activity derived from a wide variety of tissues[1–3]. This action is largely attributable to the α_2-macroglobulin component[4–7] which also binds and inhibits many other less specific proteolytic enzymes[8]. Because of its high molecular weight (725,000, ref. 9) this antiproteinase is probably excluded from many tissue sites by permeability barriers and thus the role of smaller antiproteinases may be more important in the control of enzyme activities within tissues. One such inhibitor which has been the subject of considerable study is α_1-antitrypsin, molecular weight 45,000, but the capacity of this serum protein to inhibit neutral collagenases has been in doubt[3,5,6,10,11].

We examined the inhibitory capacity of the small serum proteins against human collagenases and found that although α_1 antitrypsin was non-inhibitory a slightly smaller protein in low concentrations was capable of inhibiting collagenase activity derived from several different tissues. This serum protein of molecular weight ~ 40,000 had no effect on the activity of trypsin or papain, suggesting a certain degree of specificity. The finding of a natural inhibitor of collagenase activity, with a molecular size similar to that reported for some collagenases themselves, suggested that it might function physiologically as a regulatory factor in collagen catabolism[3,12].

The small collagenase inhibitor has been partially purified and separated from the well known serum antiproteinases α_2 macroglobulin and α_1 antitrypsin by a procedure involving gel filtration in Sephadex G-200 and G-100 superfine, with an intermediate ion exchange chromatography step using DEAE–Sephadex[3]. This preparation, when examined by Agarose electrophoresis, was shown to migrate as a single protein band corresponding to the position of a β_1 serum protein (Fig. 1). We therefore propose to call the inhibitor β_1 anticollagenase (β_1-AC). To establish the specificity of β_1-AC partially purified preparations together with purified samples of α_2 macroglobulin (α_2-M) and α_1 antitrypsin (α_1-AT) were examined for their action against rheumatoid synovial and other human collagenases, and against enzymes representative of the principal classes of proteinases.

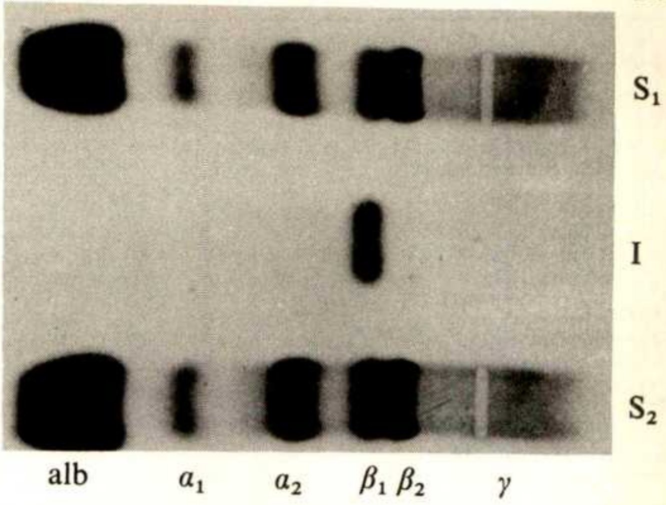

Fig. 1 Electrophoresis of the small collagenase inhibitor on Agarose. The inhibitor preparation and samples of normal human serum were applied to an Agarose film (Corning ACI) and subjected to electrophoresis for 1 h at 3 mA using 50 mM sodium barbital buffer (*p*H 8.6). The protein bands were stained with Coomassie brilliant blue. I, collagenase inhibitor; S$_1$ and S$_2$, serum samples.

At 25 °C, neutral *p*H and in the presence of calcium ions, human collagenases attack collagen molecules in solution at a single specific locus producing two products which represent three-quarter and one-quarter length fragments of the molecule[1,4]. This reaction can be monitored by viscometry and a final drop in specific viscosity of a collagen solution of approximately 55–58% usually indicates that the reaction is complete. Figure 2 shows that the addition of β_1-AC to a reaction mixture of human synovial collagenase and collagen significantly reduces the rate of

Fig. 2 Effect of β_1-AC on the reaction between rheumatoid synovial collagenase and collagen in solution determined by viscometry at 25 °C. Samples (25 µl) of synovial enzyme were added to 100-µl samples of β_1-AC (2 mg ml^{-1}) and bovine serum albumin (2 mg ml^{-1}). 100 µl of each sample was then added to separate 1 ml capacity Ostwald viscometers each containing 0.9 ml of a collagen solution. The final reaction mixtures of 1ml contained 0.4 M NaCl, 50 mM Tris-HCl buffer (*p*H 8.0) 10 mM $CaCl_2$, 0.05% arginine, 0.7 mg collagen and collagenase, with added β_1-AC protein (○) or with added bovine serum albumin as control (●). The outflow time for water at 25 °C was 28 s for both viscometers.

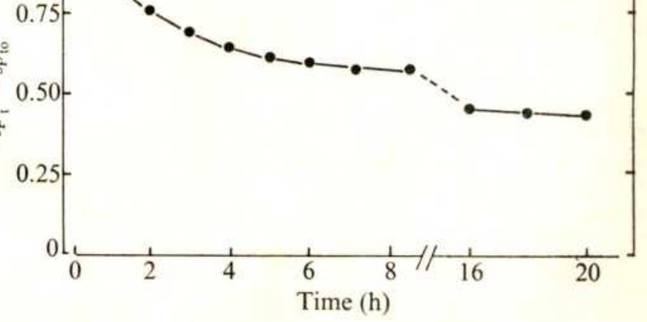

Table 1 The effect of β_1-AC on the enzymatic activity of human collagenases and various proteinases

Enzyme	Proteinase Type	Substrate	Enzyme activity Control	Enzyme activity $+\beta_1$-AC	% Inhibition
Trypsin	Serine	Casein*	1.68 (26)	1.74	0
Subtilopeptidase A	Serine	Casein	1.01 (16)	0.95	5
Elastase	Serine	Elastin-orcein†	0.19 (35)	0.20	0
Papain	Thiol	Casein‡	0.43 (7)	0.43	0
Ficin	Thiol	Casein	1.39 (22)	1.33	4
Thermolysin	Metal	Azocasein§	1.44 (22)	1.46	0
Snake venom (*Crotalus atrox*)	Metal	Azocoll‖	0.45 (18)	0.47	0
Pepsin	Carboxyl	Haemoglobin¶	0.58 (25)	0.60	0
Cathepsin D	Carboxyl	Haemoglobin**	0.32	0.30	6
Human Collagenases					
Gastric mucosal	Metal	Collagen††	1,200 (40)	240	80
Rheumatoid synovial	Metal	Collagen	1,220 (41)	731	40
Skin	Metal	Collagen	1,150 (38)	644	44
Granulocyte	Metal	Collagen	1,090 (36)	872	20

With each series of assays, control incubations containing twice the amount of enzyme were included to ensure that reactions were terminated at a point where they were still linear. Figures in parentheses shown after the control enzyme activities indicate the proportion of substrate degraded as a percentage of total available substrate determined by complete enzymatic digestion. Results presented are the means of duplicate assays after subtracting control values and are expressed as percentage inhibition of the control. *10 mg of casein dissolved in 2 ml 100 mM Sorenson's phosphate buffer (*p*H 8.0) containing 0.2 μg trypsin (Sigma, Type III) or 0.2 μg subtilopeptidase A (Sigma) was incubated for 16 h at 37 °C with and without the β_1-AC protein (40 μg ml^{-1}). Each reaction was terminated by the addition of cold 10% trichloroacetic acid, centrifuged for 20 min at 3,000*g*, and the absorbance of the supernatant measured at 280 nm. †2 mg of elastin-orcein (Sigma) suspended in 2 ml 100 mM Tris-HCl buffer (*p*H 8.8) containing 2 μg elastase (Sigma, Type III) incubated for 16 h at 37 °C with and without the β_1-AC protein (40 μg ml^{-1}). The reaction mixtures were filtered and the absorbance of the filtrates measured at 590 nm. ‡10 mg of casein dissolved in 2 ml 100 mM Sorenson's phosphate buffer (*p*H 8.0) containing 50 mM cysteine, 10 mM EDTA, 1 μg of papain or ficin (Sigma) was incubated for 16 h at 37 °C with and without β_1-AC protein (40 μg ml^{-1}). The reactions were terminated and measured as described for (*). §10 mg of Azocasein (Sigma) in 2 ml 100 mM Tris-HCl buffer (*p*H 8.0) containing 10 mM $CaCl_2$ and 0.2 μg thermolysin (Sigma protease, Type X) was incubated for 16 h at 37 °C with and without β_1-AC protein (40 μg ml^{-1}). The reactions were terminated as described in (*) and the absorbance of the supernatants measured at 366 nm. ‖5 mg of Azocoll (Calbiochem) in 2 ml 100 mM Tris-HCl buffer (*p*H 8.0) containing 10 mM $CaCl_2$ and 2 μg *Crotalus atrox* venom (Sigma) were incubated for 16 h at 37 °C with and without β_1-AC protein (40μg ml^{-1}). The reaction mixtures were filtered and the absorbance of the filtrates measured at 520 nm.

¶5 mg of haemoglobin (Sigma) dissolved in 2 ml 100 mM sodium acetate buffer (*p*H 3.2) containing 0.2 μg pepsin were incubated for 16 h at 37 °C with and without β_1-AC (40 μg ml^{-1}). The reactions were terminated and measured as described for (*). **This assay was kindly undertaken by Dr Alan Barratt of the Strangeways Research Laboratory, Cambridge, using haemoglobin as substrate[14]. ††Collagenase activity was assayed by measuring the release of soluble radioactive products from a pellet of thermally reconstituted ^{14}C-glycine-labelled collagen fibrils[12]. Each reaction mixture contained 3,000 c.p.m. and the assays were incubated for 16 h at 37 °C with and without β_1-AC (40 μg ml^{-1}).

collagen cleavage when compared with that of the control reaction.

For comparison, the effect of purified α_2-M and α_1-AT on synovial collagenase activity was also examined by viscometry and only the former was found to be inhibitory. These results confirmed previous data obtained from collagenase assays at 37 °C using thermally reconstituted ^{14}C-labelled collagen fibrils[12] indicating that the two serum proteins β_1-AC and α_2-M are potent inhibitors of human collagenase activity whereas α_1-AT is non-inhibitory.

As earlier work had shown the β_1-AC protein to have no inhibitory effect on trypsin and papain[3], studies were undertaken using nine different enzymes selected to represent the serine, thiol, metal-dependent and carboxyl proteinases, in order to obtain further information on the specificity of the inhibitor (Table 1). The serine proteinases trypsin, subtilopeptidase A and elastase, and the thiol proteinases papain and ficin were not inhibited by β_1-AC, but all were susceptible to inhibition by α_2-M. Since human collagenases are characterised as metal-dependent proteinases it was of particular interest to see if other examples of this group of enzymes were similarly inhibited by β_1-AC. Thermolysin and the snake venom from *Crotalus atrox* were selected as examples of metal-dependent proteinases but neither was inhibited by β_1-AC. The addition of β_1-AC to cathepsin D and pepsin, which are both carboxyl or acid proteinases, also failed to produce any significant inhibition.

In summary, the β_1-AC protein seems to be a specific inhibitor for human neutral collagenases such as those derived from rheumatoid synovium, gastric mucosa, skin and granulocytes. Each of these collagenases is also inhibited by purified preparations of α_2-M but not by purified α_1-AT (ref. 3).

Although the inhibitor has been characterised as a β_1-serum protein with a molecular weight of approximately 40,000, the question of whether it represents a previously characterised serum protein, or a previously unrecognised protein of very low physiological concentration must await the results of purification of β_1-AC which is in progress.

When whole serum was fractionated by gel filtration in Sephadex G-200 more than 90% of the collagenase-inhibitory capacity was demonstrable in those fractions containing α_2-M. Although the inhibitory capacity of this antiproteinase greatly exceeds that of β_1-AC in whole serum, this situation may well be reversed in certain tissue locations from which α_2-M is excluded on account of its large molecular size, its prime physiological function possibly being to protect constituents within the circulatory system from proteolytic attack.

In a condition such as rheumatoid arthritis where there exists much accumulated evidence for an important role of neutral collagenase[13] the operation of a natural inhibitor of the enzyme could be a factor in controlling the progress of the joint destruction. The finding of a small diffusible serum inhibitor, specific for collagenase, suggests that serum concentration of β_1-AC might influence the rate of collagen catabolism in this condition. This hypothesis is being investigated.

The finding that four different human collagenases of similar activity when exposed to the β_1-AC preparation showed different degrees of inhibition is of particular interest (Table 1). Although it is not yet possible to quantify this relationship on a molar basis the data suggest that each collagenase has a different susceptibility to the inhibitor, the granulocyte enzyme, for example, being far more resistant to inhibition than the gastric enzyme. Thus, alterations in the concentration of the serum inhibitor might be expected to have a differential effect on collagen breakdown in different tissues, and acquired or genetically determined inhibitor deficiencies could selectively "release" tissue collagenases at different sites. Collagen catabolism at any one site would be the net result of the local concentration of collagenase, the local concentration of the specific inhibitor, and the susceptibility of the particular enzyme to β_1-AC inhibition. Such ideas are, however, speculative at this stage.

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Are reptilian pulmonary receptors mechano- or chemosensitive?

BOTH birds and mammals possess pulmonary receptors which increase discharge in phase with each inspiration[1–5]. In mammals the adequate stimulus of these receptors seems to be the total transpulmonary pressure which varies with both inflation volume and rate and sign of the volume change[6]. The lungs of birds, however, consist of a complex system of air tubes[7,8] and unlike those of mammals are relatively inexpansible. Many avian pulmonary receptors have no mechanosensitivity, the adequate stimulus being the changes in airway CO_2 throughout the breathing cycle[9–12]. So rate and degree of inflation of the respiratory system is signalled by different sensory modalities in birds and mammals. The demonstration that mammalian pulmonary receptors are partially inhibited by high alveolar CO_2 (refs 13–15) and that some avian receptors may be mechanosensitive[16,17] suggests that in the more phylogenetically ancient vertebrate classes it might be possible to locate a pulmonary receptor which has distinct mechano- and chemosensitive properties, thereby allowing speculation on the evolution of the avian and mammalian receptor types.

We report here the responses of turtle (*Chrysemys picta*) pulmonary receptors to both mechanical and chemical stimuli. This animal was chosen because reptiles represent the evolutionary stem group for both birds and mammals. Although many reptiles or even the more phylogenetically primitive amphibia might have served, our choice was governed by two facts. First, because of their diving habits, turtles experience alveolar CO_2 levels in the mammalian range[18]; second, in possessing a chambered, saccular lung that is constrained in its expansion by the exoskeleton[19], turtles show a superficial resemblance to birds.

Turtles (600–1,200 g) were single-pithed and tidally ventilated with a constant volume, positive pressure, respiration pump. Single and multi-fibre nerve activity, in phase with artificial ventilation, was recorded in vagal slips using bipolar silver electrodes. Neural activity was amplified, visually displayed on an oscilloscope and audibly monitored. Tracheal air flow and intratracheal pressure (ITP), generated by the ventilator, were recorded with a pneumotachograph and pressure transducer respectively. All variables were stored on magnetic tape for later analysis on a Digital PDP Lab 8e mini-computer using conventional software.

Receptors with discharge modulated by artificial ventilation with air were of the slowly adapting low threshold type. Many units were active during the deflation phase when ITP approached that of the atmosphere (Fig. 1*b*), the average discharge frequency being 7.1 ± 1.4 impulses s^{-1} at end deflation. Discharge increased with lung inflation and reached its maximum at peak ITP but none of the receptors seemed to respond to the rate of change in pressure either on inflation or deflation. Discharge frequency increased with inflation by 2.09 ± 0.32 impulses per s per cm H_2O increase in ITP, and discharge was maintained when artificial ventilation was held at either end inflation or end deflation. In response to maintained inflation, only 3 of the 14 single fibres investigated showed marked adaptation which was still less than 25% when calculated from index 1 of Davis *et al.*[6]. After removal of the sternum, 6 of the 14 units were located by punctate stimulation to the major septa which divide the lung into 8–10 chambers (Fig. 1*a*) and receptor discharge patterns were shown to be unaffected by pulmonary artery occlusion.

Addition of 10% CO_2 to the ventilating gas caused a rise

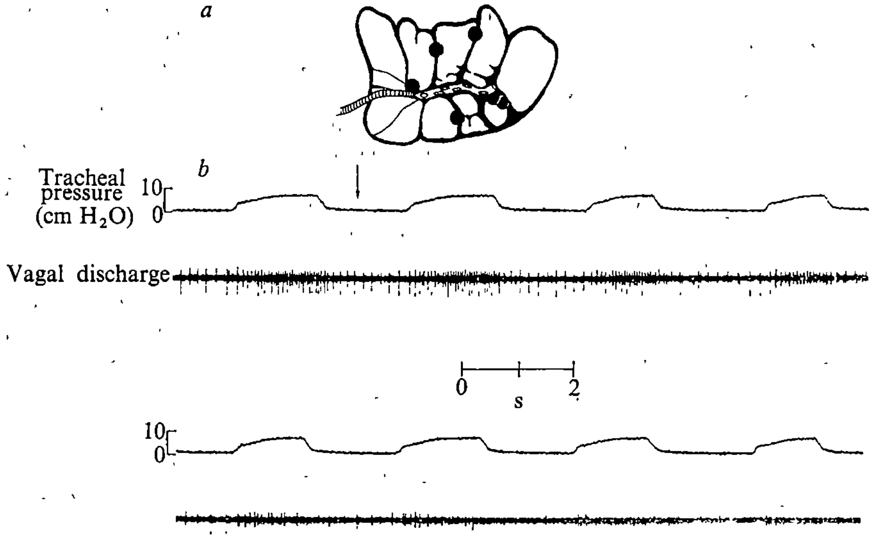

Fig. 1 *a*, Schematic diagram of the right lung of *Chrysemys picta* showing approximate site (●) of receptors located by punctate stimulation. *b*, Effect of 10% CO_2 on the discharge of a turtle's pulmonary receptors; continuous recording of intratracheal pressure (upper) and pulmonary receptor discharge (lower), 10% CO_2 introduced into the ventilating gas mixture at the arrow.

Table 1 Reduction in discharge rate of pulmonary receptors following the introduction of 10% CO_2 to the ventilating gas

Fibre no.	Peak inflation pressure (cm H_2O)	% Reduction in discharge rate associated with		
		End inflation	End deflation	One breath
1	—	33	100	66
2	5.5	46	100	65
3	7.0	27	60	26
4	4.5	63	100	68
5	6.5	100	100	100
6	6.5	100	100	100
7	9.0	37	7	27
8	9.0	56	73	52
9	9.0	53	80	45
10	7.5	36	—*	47
11	7.5	50	—*	38
12	5.0	33	30	44
13	5.0	55	60	62
14	8.0	100	—*	100

The difference between the discharge rates, when ventilating with air and 10% CO_2, is expressed as a percentage of the discharge rate with air.

*Discharge rates fell to zero while animals were breathing room air.

in threshold and a decrease in sensitivity of the receptors for any given pressure change compared with air inflation (Table 1). The increase in discharge rate on inflation was reduced to almost half (56%) the air inflation value and 3 units remained silent throughout inflation. For 5 units discharge ceased during lung deflation whereas, on the average, discharge rate during deflation was reduced by 74%. The decrease in activity began during the first inflation following a step change in CO_2 content of the ventilating gas and continued to fall over several cycles of ventilation (Fig. 1*b*) (using tidal ventilation, 2–3 breaths were required before the expired gas composition equalled that of the inspired gas). High CO_2 did not seem to affect the adaptation rate to maintained inflation. The effects of ventilating with 5% CO_2 in air were about half as pronounced as the effects of 10% CO_2. Since some units were completely silenced by ventilation with air containing 10% CO_2, any changes in lung compliance induced by the presence of CO_2 cannot explain this reduction in receptor discharge. Lung compliance, however, was decreased in four turtles whereas three showed no change in compliance when 10% CO_2 was added to the ventilating gas. In only one case did the fall in compliance (35%) match the fall in the maximum discharge frequency, at peak ITP, of the receptor being recorded.

This work has shown that although all pulmonary receptors we have monitored are mechanosensitive, some are extremely sensitive to CO_2, being completely inhibited by pulmonary concentrations of 10%. This sensitivity to CO_2 resembles that shown by avian pulmonary chemoreceptors. On the other hand, those receptors exhibiting only slight sensitivity to CO_2 have responses similar to those of mammalian pulmonary mechanoreceptors. For these reasons, the reptilian receptor type may be viewed as the functional precursor of those in higher vertebrates. It also suggests that the reflex respiratory responses to intrapulmonary CO_2 shown by reptiles[20], birds[21] and mammals[22] are phylogenetically ancient.

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Muscle activity decreases rate of degradation of α-bungarotoxin bound to extrajunctional acetylcholine receptors

DURING development of skeletal muscle, and after denervation or reinnervation of adult muscle, there are significant changes in the number of extrajunctional acetylcholine (ACh) receptors, measured both by sensitivity to ACh and by binding of α-^{125}I-bungarotoxin (for review, see ref. 1). Muscle activity has been shown to be a major factor in controlling the level of extrajunctional receptors. Direct electrical stimulation of denervated muscle both *in vivo* and *in vitro* decreases the sensitivity of the extrajunctional membrane to ACh[2–5], whereas chronic postsynaptic blockade of innervated muscle *in vivo* increases its sensitivity[6,7]. Muscle activity could alter the level of extrajunctional receptor by changing rates of synthesis or degradation of the receptor.

We have investigated the effect of direct electrical stimulation in organ culture on the number of extrajunctional ACh receptors in denervated muscles and on the degradation of α-^{125}I-bungarotoxin bound to extrajunctional receptors. We have found that the rate of toxin breakdown is slowed in conditions of stimulation which over several days reduce the number of receptors. This result suggests that activity decreases receptor levels by decreasing receptor synthesis, rather than by affecting receptor degradation.

Attempts have been made[8–11] to measure degradation of ACh receptors indirectly by binding α-^{125}I-bungarotoxin to them and monitoring the loss of radioactivity from the tissue. The loss of radioactive toxin from extrajunctional receptors in cultured rat diaphragm muscle involves degradation of toxin with release of ^{125}I-monoiodotyrosine into the medium and has many of the characteristics of intracellular protein breakdown[9]. Toxin bound to the ACh receptors of myotubes in primary cell culture is degraded by a similar process, and experiments by Devreotes and Fambrough have provided strong indirect evidence that in myotubes the rate of toxin loss reflects the normal rate of receptor turnover[11]. Although receptor breakdown has not yet been measured directly, the available evidence suggests that breakdown of receptors can be followed by measuring degradation of bound toxin.

In the present investigation, left hemidiaphragms from male white rats (50–60 g) were surgically denervated, and 5 d later, left and right hemidiaphragms were pinned to separate culture dishes coated with Sylgard, as described previously[9]. Dishes for stimulation contained platinum electrodes embedded in recessed troughs. Muscles were mounted over, but not in contact with, the electrodes and were stimulated continuously by suprathreshold pulses of

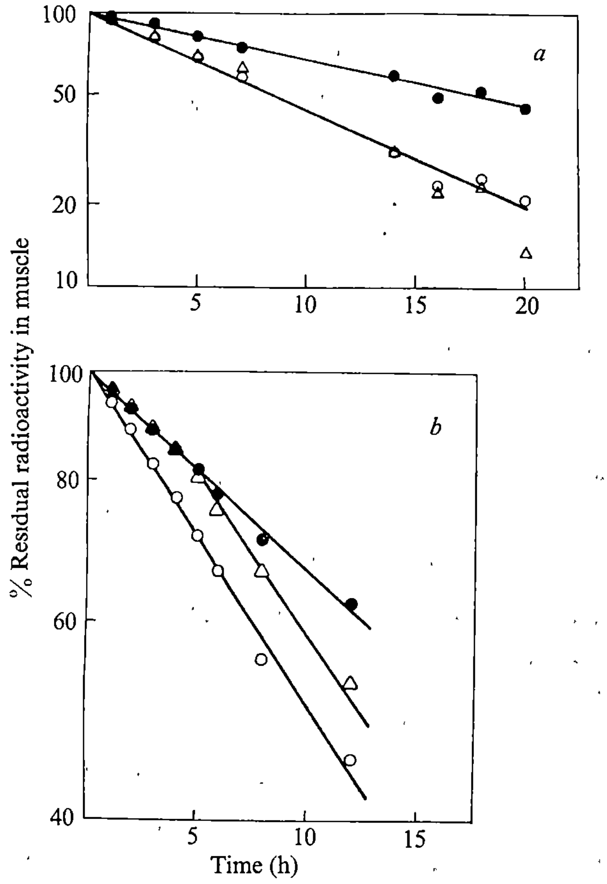

Fig. 1 Effect of muscle activity on degradation of α-^{125}I-bungarotoxin bound to extrajunctional ACh receptors in denervated muscle. Hemidiaphragms from animals that had been denervated 5 d previously were labelled *in vivo* by injection of radioactive toxin and transferred 3 h later to organ culture. After several changes of medium to reduce the level of unbound toxin in the muscle, release of radioactivity from each muscle was monitored by sampling the medium, and radioactivity remaining in each muscle at the end of the experiment was measured. Each value for the left hemidiaphragm was corrected by subtracting, on a per weight basis, the corresponding value for the right hemidiaphragm from the same animal. The total amount of radioactivity initially associated with extrajunctional receptors was calculated as the sum of the corrected values of radioactivity released into the medium plus that remaining in the muscle. Stimulation was at 2 Hz. *a*, One muscle (●) was stimulated in normal culture medium, one (△) was stimulated in culture medium containing 10^{-5} g ml^{-1} tetrodotoxin, and the other (○) was not stimulated. Duplicate 0.1-ml samples of medium were taken for each time point. *b*, All muscles were incubated for 16 h, and release was monitored during the last 12 h of the experiment. One muscle (●) was stimulated continuously, one (△) was stimulated only during the first 6 h of incubation, and the other (○) was not stimulated. Duplicate 0.5-ml samples of medium were taken for each time point.

2-ms duration and alternating polarity. In experiments testing the effects of electrical stimulation on ACh sensitivity, muscles were stimulated at a frequency of 10 Hz, comparable to the discharge frequency of single units in the phrenic nerve[5]. Muscles were removed from the incubator at the end of the culture period, and the effectiveness of stimulation through the electrodes in the dish was determined by monitoring the production of action potentials in different areas of the muscle. In general about 15 out of 20 fibres sampled gave action potentials in response to stimulation, suggesting that a high proportion of fibres had been active in culture.

Direct electrical stimulation reduced the extrajunctional sensitivity developed by denervated muscles in culture. Fibres in muscles placed in culture 5 d after denervation and cultured for 4 d with continuous electrical stimulation showed lower extrajunctional ACh sensitivities than fibres in muscles similarly treated but not stimulated (Table 1). Muscle fibres denervated at the time of culture and continuously stimulated in culture for 4 d also showed lower sensitivities than those from unstimulated muscles (Table 1). These results are similar to those of Purves and Sakmann[5], although the effects that we observe are not as large. In their experiments, stimulation was carried out for 7–8 d, and only fibres that were judged to have been continuously active were examined.

Stimulation of muscles for only 24 h in these same conditions produced no detectable change in extrajunctional sensitivity. The extrajunctional sensitivity of 5-d denervated muscles stimulated in culture for 24 h was 103 ± 10 mV nC^{-1} (mean ± s.e., $n=20$), whereas that of unstimulated muscles was 100 ± 6 mV nC^{-1} ($n=20$). In slightly different conditions of culture, Purves and Sakmann[5] reported a small decrease in extrajunctional sensitivity in muscles stimulated for 24 h.

To determine whether an increased rate of receptor degradation contributes to the observed reduction of extrajunctional sensitivity the loss of α-^{125}I-bungarotoxin bound to extrajunctional receptors was examined. Radioactive toxin was injected into the thorax of denervated rats, and 3 h later the left and right hemidiaphragms were removed and cultured as described above. The time course of loss of bound ^{125}I-toxin was followed by monitoring the appearance of radioactivity in the medium and measuring the radioactivity in the muscle at the end of the experiment. Values from right hemidiaphragms which were innervated at the time of culture were used to correct for the contribution of toxin bound to sites other than extrajunctional receptors[9].

The time course of degradation of α-^{125}I-bungarotoxin was studied in unstimulated muscles and in muscles stimulated at 2 or 10 Hz (Fig. 1). In all experiments, toxin degradation was slower in the stimulated muscles. The release of radioactivity could be described as a single exponential process whose half time ranged in unstimulated muscles from 8 to 14 h and in stimulated muscles from 16 to >30 h. When tetrodotoxin (10^{-5} g ml^{-1}) was included in the culture medium, the stimulating current did not elicit muscle activity, and the time course of loss of radioactivity from these muscles was indistinguishable from that of the unstimulated muscles (Fig. 1*a*). Thus the slower loss of radioactivity from stimulated muscle is related to activity of the fibres.

The onset of the effect of activity and its reversibility were determined in muscles stimulated during only part of the incubation. When stimulation was terminated, the rate of toxin degradation recovered to the control value within several hours (Fig. 1*b*). Conversely, a decreased rate of degradation was observed within a few hours after stimulation was begun (not shown).

Table 1 Effect of stimulation on extrajunctional ACh sensitivity of muscles in culture

	ACh sensitivity (mV nC^{-1})	
	Unstimulated	Stimulated
a, Denervated 5 d, cultured 4 d	322±35 (24)	50±9 (19)
b, Cultured 4 d	83±8 (56)	18±2 (57)

In one series of experiments denervated muscles were cultured, and either stimulated continuously at 10 Hz or not stimulated (*a*). In another series normal muscles were treated similarly (*b*). After the period in culture, extrajunctional sensitivity was determined by iontophoretic application of ACh from micropipettes that contained 2 M acetylcholine bromide and had resistances of 80–120 MΩ. No attempt was made to select fibres that had been active in culture. Values reported are mean ± s.e.m. for three pairs of denervated muscles and for eight pairs of normal muscles. In each series, the stimulated muscles showed lower sensitivity ($P < 0.001$).

The radioactivity remaining in the extrajunctional regions of stimulated muscle at the end of the experiment was extracted with detergent and examined by zone sedimentation. The sedimentation profile had a single major peak corresponding to toxin–receptor complex, and except for the larger amount of radioactivity did not differ appreciably from that obtained with extracts of unstimulated muscle. Therefore, the radioactive toxin in stimulated muscle remains bound to receptor, suggesting that stimulation may slow the process of toxin degradation at an early step.

If degradation of toxin is an adequate measure of normal receptor breakdown, the effect of activity on the rate of receptor degradation that we have observed is in the wrong direction to account for its effect on extrajunctional receptor levels. One interpretation of this result is that the effect of muscle activity on degradation may not be a specific one—since activity has been shown to decrease the average rate of protein degradation[14]—but that receptor levels may be regulated by affecting rates of synthesis. Experiments showing that denervated, but not innervated, muscles accumulate newly synthesised receptor are consistent with this idea[21,13].

We conclude that muscle activity prolongs the half life of α-^{125}I-bungarotoxin bound to extrajunctional ACh receptors in denervated rat diaphragm. This effect is reversible and occurs at low frequencies of stimulation. These findings suggest that muscle activity may slow the normal degradation of the extrajunctional ACh receptor, and they provide indirect evidence for an effect of activity on the synthesis of the ACh receptor.

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Lanthanum inhibits brain adenylate cyclase and blocks noradrenergic depression of Purkinje cell discharge independent of calcium

IT has been reported that lanthanum (La^{3+}) can reversibly antagonise the depressant effect of noradrenaline (NA) on cerebral cortical neurones[1,2]. Because this ion is known to interfere with membrane actions of calcium[3–7], it was proposed, on the basis of this and other data, that the inhibitory effects of NA are mediated through a mechanism involving transmembrane movement of Ca^{2+} (refs 1 and 2). On the other hand, evidence from other laboratories has suggested that the effects of NA are primarily related to the activation of a NA-sensitive adenylate cyclase, with a consequent increase in the intracellular concentration of cyclic AMP[8–10]. We now report that La^{3+} is a potent inhibitor of brain adenylate cyclase and suggest that this ion blocks responses of cerebellar Purkinje cells to NA by means of a mechanism involving the inhibition of NA-stimulated increases in cyclic AMP, independent of an effect on calcium.

Figure 1 (bottom curve) shows the effect of lanthanum chloride on the rate of formation of cyclic AMP from ATP in an homogenate of rat cerebellum. Enzyme activity was inhibited by concentrations of La^{3+} as low as 0.1 μM : 50% inhibition occurred at 2 μM and almost complete inhibition at 100 μM. In addition to inhibiting basal adenylate cyclase activity, La^{3+} also blocked enzyme stimulation by NA (Fig. 2*b*). In the conditions used for assaying adenylate cyclase (in which theophylline was present), phosphodiesterase (PDE) activity was largely inhibited. The little PDE activity that remained was unaffected by La^{3+}, indicating that the observed decrease in cyclic AMP formation was due to inhibition of adenylate cyclase and not to stimulation of PDE. When theophylline was omitted from the reaction medium, La^{3+} caused a small stimulation of PDE activity

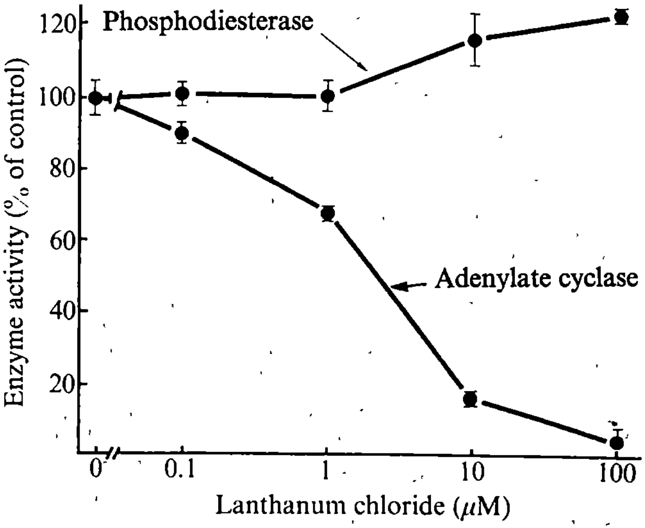

Fig. 1 Effect of lanthanum chloride on adenylate cyclase ($I_{50} = 2\ \mu$M, lower curve) activity and PDE (upper curve) activity in a homogenate of rat cerebellar cortex. The values shown are the mean (± mean deviation) of triplicate determinations. Control activities were 241±4 pmol per mg protein per min for adenylate cyclase and 16.4±0.8 pmol per mg protein per min for PDE. To measure adenylate cyclase activity, 1-mm slices of cerebellar cortex were homogenised (15 mg ml^{-1}) in 6 mM Tris-maleate buffer, *p*H 7.4. Cyclic AMP formation in these homogenates (or in particulate fractions prepared from the washed 30,000*g* pellet of the homogenate) was measured in an assay system (0.3 ml) containing 80 mM Tris-maleate, *p*H 7.4, 10 mM theophylline, 6 mM $MgSO_4$, 1.5 mM ATP and tissue (1 mg wet weight), with or without lanthanum chloride. The reaction (3 min at 30 °C) was initiated by addition of ATP, terminated by boiling for 2 min, and then centrifuged at low speed to remove insoluble material. Cyclic AMP in the supernatant was measured by the method of Brown *et al.*[29] with appropriate blanks. In some cases, samples were partially purified by ion-exchange chromatography to remove metal ions and ATP before measurement of cyclic AMP by the protein binding assay. In the experimental conditions used, enzyme activity was linear with respect to time and enzyme concentration. At concentrations less than 250 μM, La^{3+} had no effect on non-enzymatic conversion of ATP to cyclic AMP. For measurement of PDE activity, assay conditions were identical to those described above except that 0.1 μM (approximately 30 nCi) ^{3}H-cyclic AMP (New England Nuclear) was used in place of ATP, theophylline was omitted, and incubation time was 1.5 min. PDE activity was measured as the rate of formation of tritiated adenosine[30].

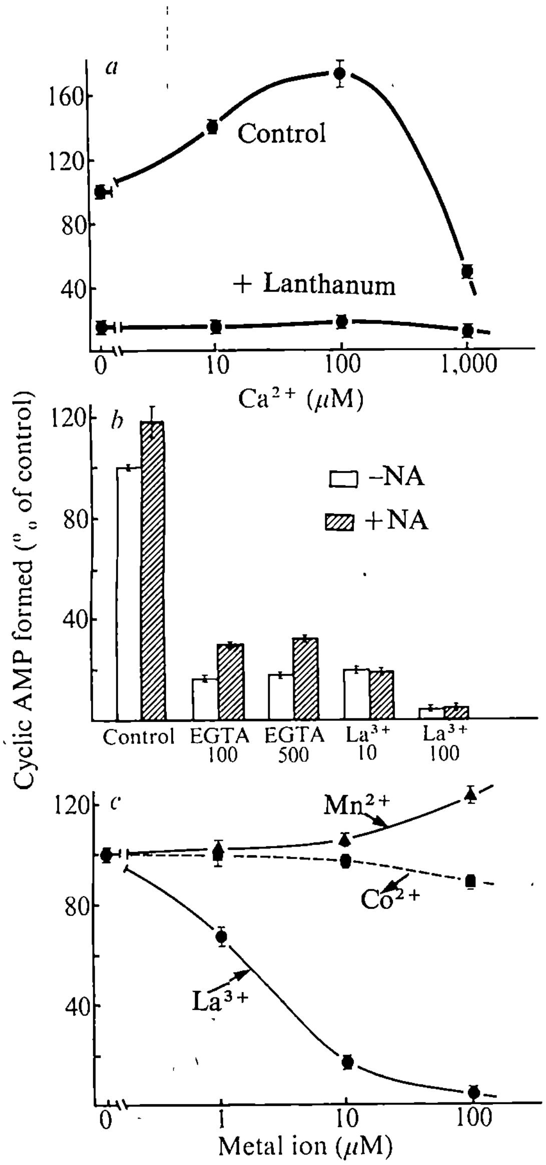

Fig. 2 Differential effects of La^{3+} and calcium on adenylate cyclase activity. *a*, Addition of exogenous calcium chloride (up to 1,000 μM) failed to reverse the inhibition of enzyme activity by 10 μM La^{3+}. Upper curve, control; lower curve, +10 μM La^{3+}. *b*, Chelation of endogenous calcium by 100 or 500 μM EGTA selectively reduced basal enzyme activity but did not affect stimulation by NA, whereas 10 or 100 μM La^{3+} inhibited basal and abolished hormone-stimulated activities Open columns, no NA; hatched columns, +250 μM NA *c*, Cobalt (■) and manganese (▲), which also interfere with membrane effects of calcium, had little inhibitory effect on adenylate cyclase The values shown in the figure are the mean (± mean deviation) of two replicate samples, each assayed for cyclic AMP content in duplicate ●, La^{3+}. Control activity in (*a*) and (*c*) was 167±3 pmol per mg protein per min and in (*b*) it was 171±2 pmol per mg protein per min.

(Fig. 1, top curve). Results similar to those in Fig. 1 were also seen when washed particulate fractions, instead of homogenates, were used as a source of enzyme. In other experiments we have observed that the lanthanide series element, neodymium, also inhibits adenylate cyclase.

Low concentrations of calcium stimulate basal adenylate cyclase activity of brain homogenates, whereas higher concentrations are inhibitory[11,12]. Also, in some non-neural tissues, certain hormone-stimulated increases in cyclic AMP depend on calcium[13]. In the experiments just described, inhibition of adenylate cyclase by La^{3+} might have been due to displacement of small amounts of endogenously bound calcium from the enzyme, preventing the usual stimulatory effect of this ion and causing an apparent inhibition of activity. For several reasons, however, we feel that such a mechanism is unlikely. First, the addition of a 100-fold excess of calcium over La^{3+} did not reverse the inhibition of adenylate cyclase (Fig. 2*a*), even though high concentrations of calcium are known to overcome partially the inhibitory effects of La^{3+} on calcium-dependent processes[6]. Second, chelation of endogenous calcium by EGTA reduced adenylate cyclase activity but did not eliminate it, whereas La^{3+} almost certainly abolished enzyme activity (Fig. 2*b*). Third, the action of La^{3+} differed qualitatively from the effect of calcium chelation, since La^{3+} reduced basal activity and totally eliminated NA-stimulated activity, whereas EGTA selectively blocked only basal activity (Fig. 2*b*). Finally, cobalt and manganese, which are also known to inhibit membrane effects of calcium[14,15], had almost no inhibitory effect on cerebellar adenylate cyclase activity at concentrations as great as 100 μM, the largest dose tested (Fig. 2*c*). Manganese actually stimulated enzyme activity to a small degree.

In electrophysiological studies we found, as observed previously[10], that most cerebellar Purkinje neurones respond to the iontophoretic application of NA with a decrease in spontaneous firing rate. Figure 3 shows that La^{3+}, applied iontophoretically during a series of NA pulses, reversibly antagonised this inhibition. In contrast, La^{3+} had no effect on the inhibition of Purkinje cell firing due to the iontophoretic application of γ-aminobutyric acid (GABA), a transmitter which does not stimulate cyclic AMP synthesis in the cerebellum[16]. La^{3+} caused strong antagonism of NA responses in nine of 11 neurones examined while not affecting responses to GABA in any of seven cells. In other preliminary experiments, La^{3+} failed to block the inhibitory actions of iontophoretically-applied cyclic AMP in three of four cells examined.

Cyclic AMP and its derivatives mimic the depressant effects of NA on most cerebellar Purkinje cells[9,17,18]. Our results demonstrate that La^{3+}, a potent inhibitor of cyclic AMP synthesis in the cerebellum, also blocks the inhibitory effects of NA on Purkinje cell discharge. Other studies have shown that exogenous La^{3+} binds to cell membranes[19,20] and therefore, *in vivo*, would have access to the regulatory subunit of NA-sensitive adenylate cyclase, also located on the plasma membrane[8]. Because stimulation of this enzyme seems to result in an accumulation of cyclic AMP in Purkinje cells[21], we propose that the NA-blocking action of La^{3+} *in vivo* is a result of direct inhibition by this ion of adenylate cyclase activity. It seems unlikely that La^{3+} acts presynaptically, since the inhibitory effects of NA seem to be the result of a direct postsynaptic action[22–25].

We have shown that certain other heavy metals, such as lead, can also inhibit brain adenylate cyclase activity, perhaps by interacting with functional sulphhydryl groups on the enzyme molecule[26,27]. Our preliminary experiments indicate that lead, too, can block the depressant effects of NA on Purkinje cell discharge. In contrast, the non-heavy metals cobalt and manganese, which at similar concentrations have little effect on adenylate cyclase activity in homogenates, also fail to block the effects of iontophoretically applied NA in Purkinje and cerebral cortical neurones[28] (In contrast to our results with the cerebellum, Yarbrough *et al.*[1,2] have reported that cobalt and manganese block NA responses of cerebral cortical neurones. Possible reasons for this discrepancy have been discussed[28].) Because cobalt and manganese, like La^{3+}, are known to interfere with the effects of calcium[14,15], calcium antagonism *per se* is not sufficient to block the NA responses of cerebellar Purkinje cells. This conclusion is supported by the observed

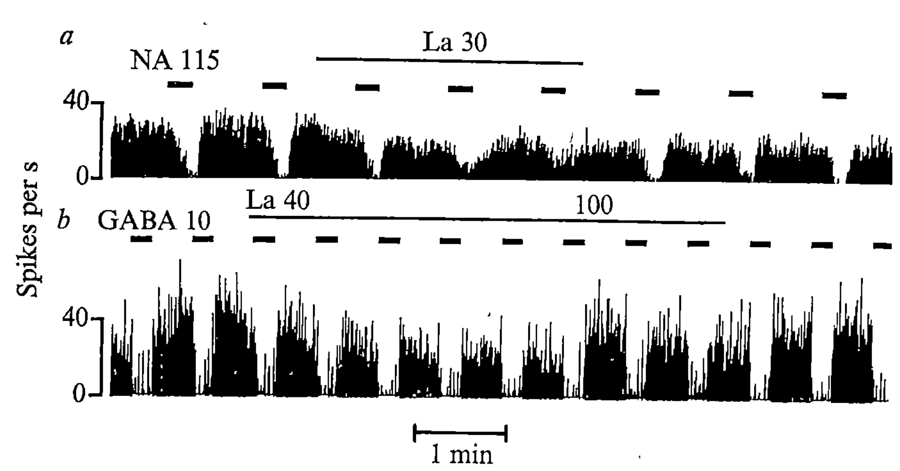

Fig. 3 Ratemeter records of Purkinje neurone discharge. *a*, Antagonism of NA-induced inhibitions by iontophoresis of La^{3+}. *b*, GABA-induced inhibitions (in a different animal) were unaffected by La^{3+}. The numbers after each drug represent the ejection current in nanoamperes, and the underlying bar is the duration of ejection. For these studies, 11 male Sprague-Dawley rats (150–200 g) were anaesthetised with 1% halothane and allowed to breathe spontaneously. Recordings were made from Purkinje cells in the vermis, lobules VI and VII, identified by anatomical location and their characteristic discharge of complex and single spikes[31]. Five-barrelled micropipettes with 4–8-µm tips were used to record extracellular action potentials of spontaneously active single Purkinje neurones and to apply substances at the site of recording by micro-iontophoresis. The centre recording barrel was filled with 5 M NaCl; three side barrels were filled with drugs by centrifugation. The remaining side barrel, filled with 3 M NaCl, was used to balance drug ejection and holding currents by automatically passing an equal and opposite charge. Drug solutions were: 0.5 M l-NA HCl, *p*H 4.5; 1.0 M GABA, *p*H 40; 0.25 M cyclic AMP, *p*H 7.5; 0.2 M LaCl, *p*H 7.4 in 0.1 M NaCl. A crystal clock controlled the iontophoresis circuit[32], so that pulses of uniform current and duration could be applied at equal intervals[28]. Iontophoretic artefacts of current, drug *p*H, and local anaesthesia were controlled carefully, as before[18]. For each cell studied, La^{3+} was tested at least twice against agonist responses.. Data from cells which did not completely recover the agonist response after cessation of La^{3+} application were discarded[28].

failure of exogenous calcium to overcome La^{3+} inhibition of adenylate cyclase *in vitro* and by the qualitatively and quantitatively different effects of La^{3+} and EGTA. Thus although the results presented here do not diminish the role of La^{3+} as a calcium antagonist or preclude some indirect interaction of La^{3+} and calcium on cyclic AMP metabolism in the intact Purkinje cell, they indicate that La^{3+} has potent effects on other biochemical mechanisms which may be involved in synaptic transmission.

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Actions of a dopamine analogue and a neuroleptic at a neuroglandular synapse

Substances which mimic or inhibit the actions of dopamine in the vertebrate central nervous system (CNS) have become of increasing interest since the discovery of their potential clinical importance[1]. It has been suggested that the receptors involved are closely associated with an adenylate cyclase responsible for the formation of cyclic AMP by homogenates of the neostriatum and limbic regions of the brain in the presence of dopamine[2]. In favour of this idea, substances believed to be agonists for central dopamine receptors also stimulate the production of cyclic AMP in such homogenates[2,3], and neuroleptics, which are believed to act by blocking dopaminergic transmission, inhibit the action of dopamine on homogenates[2,4]. It is difficult, however, to obtain information about the physiological mode of action of these compounds at the cellular level in the CNS. We report here the effects of a dopamine-like agonist and a neuroleptic on an alternative system which enables investigation of intracellular responses. Our results are consistent with the idea that both substances act directly on postsynaptic dopamine receptors.

The substances tested were 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) which, as predicted by Woodruff[5], has been found to be an analogue of dopamine both physiologically[6] and biochemically[3]; and flupenthixol, of which the *cis* isomer (α-flupenthixol) is a potent neuroleptic[7] and inhibitor of the stimulation of cyclic AMP formation by dopamine, whereas the *trans* isomer (β-flupenthixol) is inactive[4]. The preparation used consisted of the innervated cockroach salivary gland which contains acinar cells responsive to dopamine and for which there is evidence of dopaminergic transmission[8,9] Both externally applied dopamine and nerve stimulation produce hyperpolarisations.

Responses to nerve stimulation and to ADTN are shown in Fig. 1*a*. A substantial hyperpolarisation was obtained on application of less than 1 μM ADTN. Figure 1*b* illustrates the progressive inhibition by 0.5 μM α-

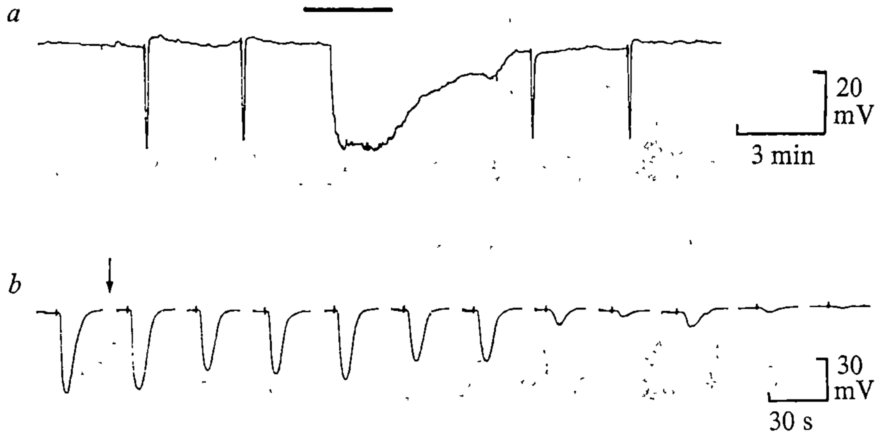

Fig. 1 *a* and *b*, Intracellular records from two cockroach salivary gland cells. The unlabelled downward deflections are hyperpolarisations in response to short trains of stimuli delivered at about 3-min intervals to the salivary duct nerve at a frequency of 100 Hz. *a*, Bar indicates the period during which 0.75 μM ADTN was present in the bathing solution flowing through the bath; *b*, 0.5 μM α-flupenthixol was added after the arrow; the block developed progressively. The preparation is described in ref. 11.

flupenthixol of successive responses to nerve stimulation. In Fig. 2 the action of α-flupenthixol is shown both on responses to applied dopamine and on approximately matching responses to nerve stimulation. The dopamine responses were reduced to a somewhat greater extent; this does not support the view that the effect of α-flupenthixol is predominantly presynaptic[10].

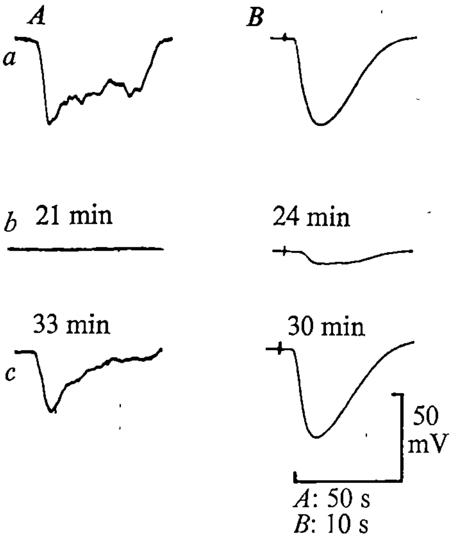

Fig. 2 Intracellular records from a cockroach salivary gland cell. Downward deflections are hyperpolarisations. Responses to dopamine (*A*) and to trains of nerve stimuli (*B*) before (*a*) and in the presence of (*b* and *c*) 0.5 μM α-flupenthixol. *a* and *b*, Concentration of dopamine was 0.18 μM and the train consisted of 4 stimuli at 100 Hz. *c*, Concentration of dopamine was raised to 2 μM and the number of stimuli to 10; the responses show that the blockade is at least partly surmountable. *b* and *c*, Time for which the α-flupenthixol had been present is shown in each record.

Our results show that at this synapse ADTN is a potent agonist and α-flupenthixol a potent antagonist. they thus reinforce the analogy between the electrophysiological effects of dopamine and its ability to stimulate cyclic AMP production.

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Plasma noradrenaline increases with age

We measured plasma noradrenaline (NA) levels in about 20 individuals who were to serve as normal control subjects and noted that older subjects tended to have higher NA levels. Extending the study to teenage and elderly subjects revealed that basal levels of plasma NA correlate with age and that the increase in plasma NA in response to stress is similarly related to age. There is considerable evidence that sensitivity to NA and NA metabolism change with increasing age. In rabbits and cats the threshold for cardiovascular response to low levels of NA decreases with old age[1]. In ageing rats uptake of NA into the heart is greater than in young animals[2] and there is a diminished inotropic response of aged rat myocardium to a fixed concentration of NA (ref. 3). Cardiac monoamine oxidase activity increases severalfold during the life span of a rat while dopa decarboxylase decreases during the first year[2]. In man, propranalol, which blocks β-adrenergic receptors, reduces heart rate and cardiac output during exercise, but this effect is considerably smaller in older subjects[4]. The response of heart rate to hypoxia and hypercapnia is attenuated in older men[5].

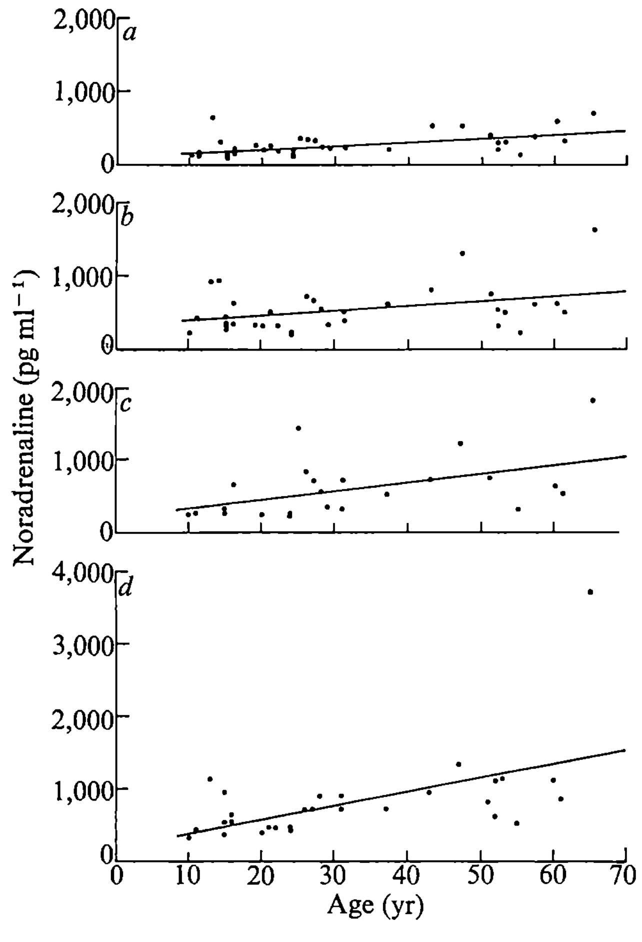
The enzyme that synthesises NA, dopamine-β-hydroxylase, is secreted along with NA during sympathetic nerve activity[6]. Blood levels of dopamine-β-hydroxylase increase with age[7]. Vanillymandelic acid, a major metabolite of NA, is excreted in increasing amounts through childhood and adolescence[8].

The possible relationship of blood levels of NA to age is important in clinical studies; several investigators have reported elevated plasma levels of NA in essential hypertension[9–16], but the ages of the patients with hypertension may have been different from the control subjects.

We measured NA in normotensive subjects, in good health, ranging from 10 to 65 years of age. None was taking medication and all were asked to abstain from coffee, tea, cola, cocoa and cigarettes before testing but otherwise followed their normal diet and activity. The testing procedure and its purpose were thoroughly explained to all subjects and written consent obtained. They were asked to lie supine and the needle of a "heparin lock" was placed in an antecubital vein of their non-dominant arm and after at least 20 min of rest a 12-ml blood sample was taken. We had previously determined that noradrenaline levels are consistently higher immediately following venepuncture than after a 20-min rest, but do not decrease further for 3 h. Subjects were then asked to stand and a blood sample obtained after 5 and 10 min. Most subjects were then asked to squeeze a hand dynamometer as hard as possible (Jamar Dynamometer, Asimow) and to maintain 30% of the maximum force for 5 min (ref. 17) at which time a final blood sample was obtained. Pulse and blood pressure were measured while subjects were lying and standing and the pulse rate was measured during the last minute of exercise. Blood samples were mixed with ACD anticoagulant, stored on ice and centrifuged within 30 min, the plasma separated and stored at -100 °C until assayed. NA was measured by a modification of the method of Henry *et al.*[18] which uses the conversion of NA to ^{3}H-adrenaline by phenylethanolamine-*N*-methyl-transferase catalysed transfer of a ^{3}H-methyl group from ^{3}H-*S*-adenosylmethionine. The assay could detect 20 pg ml^{-1} of NA.

The mean resting blood pressure of these subjects was 115/70 and after standing 5 min this changed to 112/75 and after 10 min was 112/78. The mean heart rate of these subjects increased from a resting level of 73 beats min^{-1} to 89 after they stood for 5 min and 87 after standing for 10 min. Five minutes of isometric exercise increased their heart rate to 106 beats min^{-1}. Normotension was a criterion for entry into this study and in these subjects resting systolic blood pressure ranged from 95 to 135 and diastolic pressure from 50 to 85; neither were related to age. There was, however, a significant correlation between plasma NA levels and age (Fig. 1). This correlation was apparent whether the subjects were recumbent, standing for 5 or 10 min or performing isometric exercise. The correlation of NA with age was about $r=0.5$ for all postures and exercise. Erect posture and exercise evoked a greater increase in plasma NA levels in older subjects; thus the slope of the regression lines in Fig. 1 are steeper for those manoeuvres resulting in the highest NA levels. During exercise there was an increase in heart rate by 34 (± 3) beats min^{-1} (s.e.m.), but there was no significant correlation of this increase with age ($r=0.04$). In older individuals, however, there was a greater increase in NA levels during exercise and the increase in NA levels between rest and exercise significantly correlates with age ($r=0.48$, $P<0.01$). Older subjects have higher basal levels of plasma NA and those subjects with high basal NA levels have a larger increase in their NA levels after exercise. Thus the absolute increase in NA levels after exercise correlates with age but the NA increase expressed as a percentage of the resting level does not ($r=0.01$). Although plasma NA levels after exercise are quite variable (coefficient of variance = 101%), when expressed as a percentage increase over the resting NA level the increments are less varied (coefficient of variance = 58%) because those individuals with the highest basal NA levels tend to have the largest NA increases.

Fig. 1 Plasma noradrenaline in pg ml^{-1} of subjects aged 10 to 65 yr while supine and resting (*a*), standing for 5 (*b*) or 10 (*c*) min or performing isometric exercise (*d*).



Of the 38 individuals in this study, seven complained of feeling faint or dizzy while standing. Some had a frank vasovagal reaction with yawning, bradycardia and pallor and could not continue the test procedure. These subjects were all under the age of 25, and had basal NA levels ranging from 130 to 650 pg ml^{-1}; when correlations of NA with age are made without these individuals the correlation coefficients are at least as high as when they are included.

The 16 males in this study were aged from 10 to 65 and had a mean age of 30.3 ± 4.0 yr, and a mean resting plasma NA level of 270 ± 42 pg ml^{-1} ($\pm$s.e.m.). The 22 females were aged from 11 to 61 and had a mean age of 30.6 ± 3.7 yr, and NA of 307 ± 31 pg ml^{-1} ($\pm$s.e.m.). The difference in plasma NA levels between males and females is not significant.

Plasma levels of NA gradually increase with age in resting subjects and older subjects have a greater increase in plasma NA levels in response to a uniform stress. These observations may be attributed to increased secretion of NA or to decreased catabolism or reuptake of the secreted NA in older subjects. Increased secretion of NA should lead to increased excretion of urinary metabolites of NA, and vanillymandelic acid is excreted in increasing amounts from birth to age 20 (ref. 8). Rat myocardium becomes less responsive to the effects of NA with increasing age because of NA receptor insensitivity[3]. Decreased responsiveness to the effects of NA in man might lead to a compensatory increased NA secretion during rest and in response to stress. Older subjects have a diminished response to β blockade with propranalol[4] and this may be a reflection of decreased adrenergic receptor sensitivity with age. If the sensitivity of adrenergic receptors does diminish with age, then the response not only to propranalol but to many other noradrenergic agonist and antagonist drugs may diminish with increasing age.

Although the incidence of hypertension increases with age, in most reports of elevated plasma levels of NA in hypertension the control subjects have not been age-matched. These observations suggest that in studies of plasma levels of NA or of responsivity of the sympathetic nervous system to stress and drugs, age should be considered in choosing appropriate control subjects.

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Depressed cardiac cyclic GMP-dependent protein kinase in spontaneously hypertensive rats and its further depression by guanethidine

BIOCHEMICAL modifications in cardiac hyptertrophy, secondary to high blood pressure, in spontaneously hypertensive (SH) rats include reduced receptor reactivities to isoproterenol[1,2] and glucagon[3], and lower contents of cyclic AMP and cyclic GMP due to aberrations in adenylate and guanylate cyclases or phosphodiesterases, or both. Also possibly affected are cyclic AMP-dependent protein kinase (A-PK) and cyclic GMP-dependent protein kinase (G-PK), enzymes that presumably mediate the effects of the respective cyclic nucleotide *in vivo*. An accurate assay for G-PK from mammalian tissues is possible in the presence of stimulatory modulator (specifically stimulating G-PK), either as a separated and purified form[4] or as a mixture with inhibitory modulator (specifically inhibiting A-PK) in a form of crude protein kinase modulator[5,6]. We have examined possible involvements of cardiac G-PK, relative to A-PK, in chronic hypertension and possible effects of guanethidine, a hypotensive drug, on the two key enzymes. We found that the cardiac level of G-PK in spontaneously hypertensive rats was lower than that in normotensive rats, whereas that of A-PK was the same in both groups. Guanethidine depressed the former enzyme further without affecting the latter.

Breeding pairs of SH (SH/N) and normotensive (WKY/N) rats were obtained from the NIH. WKY/N are descendants of the original Kyoto Wistar colony from which the SH mutant was bred by Okamoto[7].

There was less protein kinase activated by cyclic GMP in extracts of heart ventricles and atria from SH rats than in those from normotensive rats, whereas the contents of the enzyme activated by cyclic AMP were the same in both groups (Table 1). Since the concentration (0.3 μM) of either cyclic nucleotide used was such that they activate preferentially the respective protein kinases to a near maximal extent without significantly ($< 5\%$) cross activating the others[5,6], we interpret the data as evidence that the myocardial levels of soluble G-PK were specifically depressed in SH rats. For comparison, we measured the contents of enzymes activated by cyclic AMP and cyclic GMP in lung extracts from both groups: they were the same. Treatment of SH rats with guanethidine, a drug that depletes catecholamines from peripheral storage, reduced systolic blood pressure and the ratio of heart (ventricle) to body weight due to decreases in cardiac size (Table 2). The drug treatment unexpectedly caused a further depression of the enzyme activated by cyclic GMP, whereas that activated by cyclic AMP remained practically unchanged. The contents of the enzymes in the particulate fraction (30,000*g* pellet), activated either by cyclic GMP or cyclic AMP, were very low and not affected by hypertension or the drug.

To ascertain that the observations made with crude extracts (Tables 1 and 2) truly reflect alterations in the enzyme levels, G-PK and A-PK in extracts were separated by isoelectric precipitation[6,8]. Table 3 shows that the myocardial levels of G-PK in SH rats were indeed lower than those in normotensive rats; guanethidine further depressed G-PK, accompanied by decreases in blood pressure. A-PK, on the other hand, was not depressed in SH rats, nor was it affected by the drug. It is interesting that the drug did not affect significantly the G-PK or A-PK levels in the extracts of heart from normotensive rats. The contents of both classes of protein kinases in the extracts of lung of SH and normotensive rats were not affected by the drug (data not shown).

Table 1 Contents of protein kinases stimulated by cyclic GMP and cyclic AMP in extracts of the heart ventricle and atrium and the lung from normotensive and SH rats

Rat and tissue	Content of basal enzyme activity¶	% Stimulation by		Ratio of stimulation by cyclic GMP to cyclic AMP
		Cyclic GMP	Cyclic AMP	
Heart (ventricle)				
Normotensive (7)	43±8	91±4	236±8	0.38±0.06
SH (7)	45±12	24±2*	230±7	0.10±0.02*
Heart (atrium)				
Normotensive (7)	52±6	80±7	129±22	0.62±0.05
SH (7)	56±8	33±8*	115±16	0.29±0.02*
Lung				
Normotensive (7)	125±12	105±10	196±12	0.54±0.04
SH (6)	132±15	109±13	195±16	0.56±0.06

Systolic blood pressures, measured by the tail cuff method (Arteriosonde 1010, Roche), for the normotensive rat (male, 8 months old) and SH rat (male, 8 months old) were 126±6 and 220±12 mmHg, respectively. The ratios of ventricle to body weight for the normotensive and SH rats were 0.0028±0.0002 and 0.0041±0.0001, respectively. The rat tissues were homogenised in 10 volumes of ice-cold 50 mM potassium phosphate buffer (*p*H 7.0), using glass–Teflon homogenisers. Crude extracts were obtained by centrifuging the homogenates for 20 min at 30,000*g*. The standard assay system[4,5] for protein kinases contained, in a final volume of 0.2 ml, potassium phosphate buffer (*p*H 7.0), 10 μmol; theophylline, 0.5 μmol; arginine-rich histone (HA, Worthington), 40 μg; $MgCl_2$, 2 μmol; γ-^{32}P ATP, 1 nmol, containing about 1.1×10^6 c.p.m.; either cyclic AMP or cyclic GMP, 60 pmol; and appropriate amounts (10–28 μg) of protein kinase fractions. Crude protein kinase modulator (50 μg protein) from rat liver was added to the incubation mixture when the cyclic GMP-stimulated enzyme activity (G-PK) was measured, whereas it was omitted when the cyclic AMP-stimulated activity (A-PK) was measured. The reaction was carried out for 10 min at 30 °C. One unit of enzyme is that amount of enzyme activity that transferred 1 pmol of ^{32}P from γ-^{32}P ATP to histone in the assay conditions.

¶Enzyme activity is expressed as U per mg protein in extracts. Numbers of rats used are given in parentheses.

*Significantly different from the normotensive rats ($P < 0.005$).

Table 2 Effects of guanethidine on contents of protein kinases in ventricular extracts from SH rats

Guanethidine treatment	Blood pressure (mmHg)	Ratio of ventricle to body weight	Content of basal enzyme activity¶	% Stimulation by Cyclic GMP	% Stimulation by Cyclic AMP	Ratio of stimulation by cyclic GMP to cyclic AMP
Experiment 1						
Untreated (4)	213±8	0.0041±0.0001	48±2	27±3	197±9	0.14±0.01
Treated (4)	162±9*	0.0036±0.0001*	49±2	12±2†	195±13	0.06±0.01†
Experiment 2						
Untreated (5)	189±4	0.0040±0.0001	67±4	26±2	215±26	0.12±0.02
Treated (5)	152±3*	0.0036±0.0001†	75±8	11±3†	207±29	0.05±0.01†

Male SH rats (8 months old) and female SH rats (7 months old) were used for experiments 1 and 2, respectively. Treated rats were given guanethidine (25 mg d^{-1}) orally for 14–19 d, and the rats were killed 24 h after the last drug administration. Assay conditions were as in Table 1.

¶Enzyme activity is expressed as U per mg protein in extracts. Numbers of rats used are given in parentheses.

*Significantly different from the non-treated group ($P < 0.001$).

†Significantly different from the non-treated group ($P < 0.005$).

Table 3 Levels of soluble G-PK and A-PK in the heart ventricle of normotensive and SH rats with or without guanethidine treatment

Rat and guanethidine treatment	Blood pressure (mmHg)	Protein kinase activity (U × 10^{-3} per g tissue)					
		*p*H 5.3-precipitate			*p*H 5.3-supernatant		
		None	Cyclic GMP	Cyclic AMP	None	Cyclic GMP	Cyclic AMP
Normotensive rats							
Untreated (5)	122±6	0.7±0.1	2.8±0.3	0.8±0.2	1.6±0.2	2.2±0.2	8.1±0.6
Treated (5)	108±6	0.6±0.1	2.5±0.2	0.8±0.2	1.4±0.1	2.1±0.3	9.5±1.2
SH rats							
Untreated (5)	218±12*	0.4±0.1	1.2±0.2*	0.5±0.1	1.5±0.3	2.5±0.5	8.6±0.7
Treated (5)	152±10†	0.2±0.1	0.6±0.1†	0.3±0.1	1.7±0.2	2.6±0.4	8.5±0.6

Male rats (8 months old) were used in each group. Treated rats were given guanethidine (50 mg d^{-1}) orally for 16 d, and killed 24 h after the last drug administration. G-PK (*p*H 5.3-precipitate) was assayed in the presence of crude protein kinase modulator (50 μg) whereas A-PK (*p*H 5.3-supernatant) was assayed in its absence.

Numbers of rats used are given in parentheses

*Significantly different from the untreated normotensive rats ($P < 0.005$).

†Significantly different from the untreated hypertensive rats ($P < 0.01$).

With perfused hearts[9] and heart slices[10], cyclic AMP and cyclic GMP may mediate the cardiac stimulatory and inhibitory effects of catecholamines and acetylcholine, respectively. In a state of sustained elevated blood pressure, the heart adapts to work more efficiently. Physiologically, this manifests itself as compensatory hypertrophy. Biochemically, a decreased ratio of the effects mediated by cyclic GMP to those mediated by cyclic AMP may be important. This can be accomplished, at least in part, by either depressing the G-PK levels, or increasing the A-PK levels, or both. Our findings indicate that only the depressed G-PK levels were associated with the cardiac hypertrophy; this may be of some biological significance, since A-PK is present at much higher levels than G-PK and consequently it would require a great deal of the newly synthesised A-PK to lower the enzyme ratio significantly. The effect of guanethidine in further depressing the G-PK levels points to an interesting mode of action of the drug—it may facilitate the compensatory mechanism of the heart. This particular effect of the drug seems to persist even after the hypertension and cardiac hypertrophy of SH rats were largely corrected.

We are investigating whether depression of cardiac G-PK can also be seen in non-genetic forms of chronic hypertension, such as those induced by stress[11] and combined administration of deoxycorticosterone and salt[12]. It will also be interesting to study the effects of other types of hypotensive drugs, such as minoxidil (a directly acting vasodilator) and clonidine (a centrally acting drug), to determine whether they also cause a further depression of cardiac G-PK in SH rats.

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Possible role for cysteine biosynthesis in conversion from mycelial to yeast form of *Histoplasma capsulatum*

Histoplasma capsulatum is a pathogenic dimorphic fungus which causes histoplasmosis in animals and man. The free-living saprobic form found in soil is mycelial, but, on infection, or in the laboratory, when the temperature is

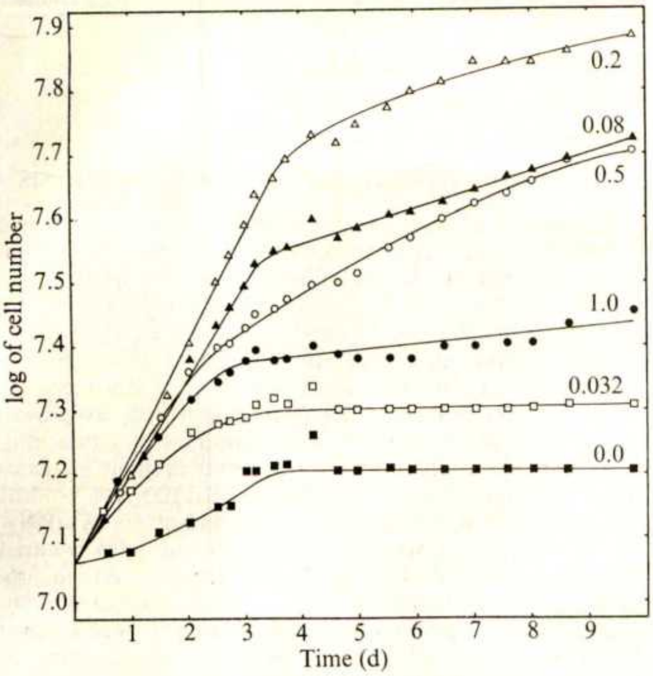


Fig. 1 Effect of increasing L-cysteine concentration on the growth of the yeast phase cells. *Histoplasma capsulatum*, Downs strain, mating type (−), was used throughout this study. Yeast cells (approximately 10^7 cells per ml) were suspended in 50 ml of basal minimal medium (2% glucose, 0.7% Difco yeast nitrogen base without amino acids) in 300-ml triple-baffled flasks with side arms (Bellco). Cysteine was added to final concentrations (mM) as indicated in the graph. The flasks were incubated at 37 °C in a New Brunswick G25 gyratory incubator shaker. The cell growth (turbidity) was measured at 600 nm in a Spectronic 20 colorimeter (Bausch and Lomb).

raised from 23 to 37 °C, it converts reversibly to a unicellular, parasitic yeast-like form. Little is known about the biochemistry of this process. There have been reports of quantitative and qualitative differences in the chemical composition of the two forms[1,2], and of changes in RNA metabolism during the conversion[3]. Also, whereas extracts from the yeast phase cells contain multiple species of RNA polymerase, those from mycelia have only traces of activity, but contain an inhibitor of RNA polymerase[4]. The yeast and mycelial cells differ in sensitivity to amphotericin B (a polyene) and actinomycin D[5], but there is no significant enzymatic difference between the two phases with respect to the glycolytic pathway and the tricarboxylic acid cycle[6]. The initiation of conversion requires a temperature change and also low oxidation–reduction potential (O/R) of the growth medium[7], but the maintenance of the yeast phase requires sulphur-containing organic compounds, especially cysteine[8,9]. The possibility of a nutritional requirement for –SH groups has been suggested[10] but not explored. We now report that the yeast cells lack the enzyme sulphite reductase so that they cannot reduce inorganic sulphite to sulphide, and thus cannot synthesise cysteine. In contrast, mycelial cells, with active reductase, can grow without cysteine supplement.

Figure 1 shows the growth curves obtained for the yeast cells in liquid basal minimal medium to which increasing amounts of L-cysteine were added. At the optimal cysteine concentration (0.2 mM) the generation time in basal medium was about 41 h, about twice as long as in a complex GYE medium (2% glucose, 1% Difco yeast extract). At concentrations higher than 0.2 mM cysteine had a strong inhibitory effect on growth. This could be due to the presence of an excess of reducing groups, or to inhibition of a stage in serine biosynthesis, either by repression or feedback inhibition. This supposition is based on the observed stimulatory effect of serine on yeast growth in the presence of cysteine, although serine alone does not support the growth (data not shown).

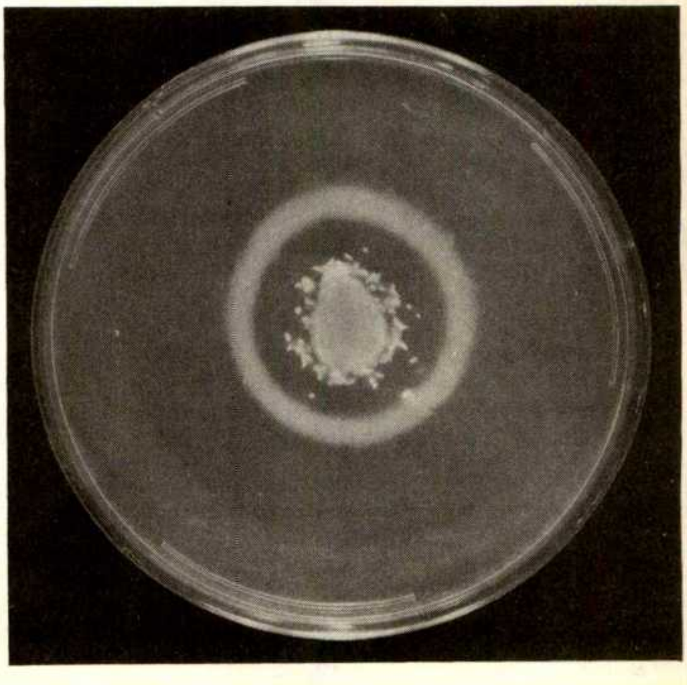
The requirement for cysteine could be satisfied by supplying the cells with Na_2S (Fig. 2). Sodium sulphide is toxic at a high concentration because of the formation of sodium hydroxide when it dissolves. This accounts for the lack of growth and precipitation of cell debris (and also of MgO from the medium) at the point of application of Na_2S crystals to the plate. At a more permissive concentration, however, the sulphide will allow a thick zone of growth to appear.

Cell-free extracts of mycelial and yeast phases were prepared as described before[11], and assayed at 37 °C for the ability to reduce SO_3^{2-} (sulphite) to H_2S (hydrogen sulphide). As Fig. 3 shows, the reaction in mycelial extract proceeded in linear fashion with time. In contrast, the yeast extract showed no detectable activity even after 90 min of incubation. Similarly, an increase in protein concentration in the assay resulted in a linear increase in the amount of H_2S produced by mycelial extract, but no activity could be seen in the yeast extract. Lowering the temperature of the assay to 23 °C did not result in the appearance of sulphite reductase activity in the yeast material.

The sulphite reductase activity observed in mycelium was low, about 50–70 times lower than that found in bacterial cells such as *Desulfovibrio vulgaris* (not shown). Nevertheless, the mycelial form of *H. capsulatum* can grow, albeit slowly, on minimal medium without added cysteine. Cysteine stimulates mycelial growth, but its effect is not great. Therefore the slowness of the growth must be due to some other unidentified factor.

It is not entirely surprising that the yeast phase form of *H. capsulatum* is less self-sufficient than the mycelial form. In an infected animal or man all nutrients are available for the fungus to thrive on. It is surprising, however, that the metabolic block is apparently limited to one pathway, and

Fig. 2 Effect of sodium sulphide on the growth of the yeast phase cells. Yeast cells (2×10^7 cells per plate) were plated in a layer of 0.7% agar on a basal minimal medium containing 2% agar. The total solid medium volume in the plate was about 30 ml. A crystal of Na_2S was deposited in the centre of the plate on top of the soft agar layer, and allowed to dissolve and to diffuse into the agar. Almost immediately after the application, small crystals of magnesium oxide started to appear. The plate was taped with Blenderm surgical tape (3M Company), inverted, and incubated at 37 °C for 14 d.



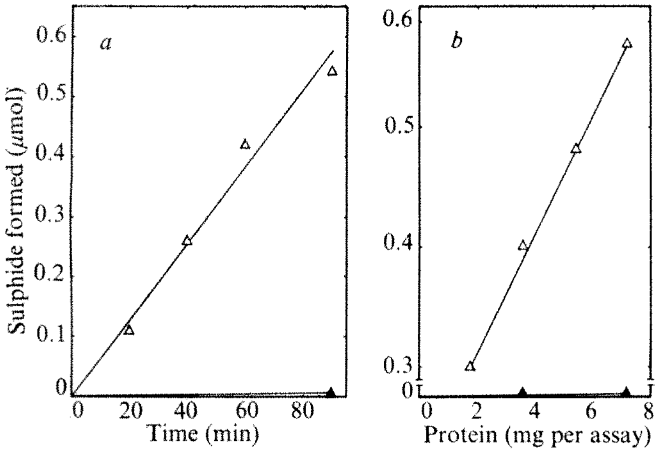


Fig. 3 Sulphite reductase activity in extracts from the mycelial and yeast phase of *H. capsulatum*. Yeast and mycelial cells were grown and extracts prepared as described before[11]. The extracts (▲, yeast; △, mycelium), containing 10–12 mg protein per ml, were used without further purification for the sulphite reductase assay[12] modified by using Tris buffer, *p*H 8.0, instead of phosphate buffer. *a*, Activity as a function of time (4.0 mg protein per assay); *b*, activity as a function of protein concentration (time of assay: 60 min.).

one enzyme in that pathway. The lack of sulphite reductase activity in the yeast cells cannot be explained by the thermal instability of the enzyme in the conditions of the yeast phase growth (37 °C). The enzyme from mycelium functions even better at 37 than at 23 °C. Also, the presence in the yeast extract of an inhibitor of sulphite reductase can be excluded because mixing of the yeast and mycelial extracts had no effect on the activity (not shown). It is tempting to hypothesise that the environmental change (temperature rise, drop in the oxidation–reduction potential) triggers biochemical change resulting in a selective shutting-off of a gene for sulphite reductase biosynthesis. The exact mechanism by which the gene inactivation could occur is unknown. One experimental approach to elucidate this mechanism would be to isolate mycelial and yeast mutants unable to undergo morphological transition. The search for such mutants has already been initiated in our laboratory.

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Increased mitochondrial leucyl- and phenylalanyl-tRNA synthetase activity as a result of inhibition of mitochondrial protein synthesis

THE synthesis of certain mitochondrial proteins is inhibited by cycloheximide, an inhibitor of cytoplasmic protein synthesis, and not by the inhibitors of mitochondrial protein synthesis, chloramphenicol and ethidium bromide[1,2]. This suggests that these proteins are synthesised on cytoplasmic ribosomes from messenger RNA (mRNA) transcribed in the nucleus. Their formation, however, is dependent, in some way, on mitochondrial protein synthesis, as growth in the presence of inhibiting concentrations of either ethidium bromide or chloramphenicol results in great increases in the specific activity of mitochondrial DNA-dependent RNA polymerase[1], elongation factors, and methionyl-tRNA transformylase, as well as an increase in mitochondrial ribosomal proteins[2]. This led Barath and Küntzel[1] to propose that the increased synthesis of mitochondrial proteins results from a disruption of mitochondrial control over transcription of nuclear genes specifying the structure of mitochondrial proteins. The model proposes a repressor protein, specified and produced by the mitochondrion, that regulates transcription of mitochondrion-specific genes in the nucleus. When synthesis of the putative repressor is inhibited by either ethidium bromide or chloramphenicol, the target nuclear genes are transcribed at an increased rate and the production of mitochondrial proteins increases. Although a direct test of Barath and Küntzel's model would involve identifying the repressor and its targets, some of the underlying assumptions may be checked by measuring the production by *Neurospora* of the mitochondrial leucyl-tRNA synthetase, whose structure is known to be determined by a nuclear gene in the *Leu*-5 region of linkage group V (refs 3 and 4, and our unpublished results). In this case the site of mRNA synthesis is deduced from formal genetic analysis rather than from patterns of inhibition by inhibitors of protein synthesis. As an internal control we also followed the synthesis of the mitochondrial phenylalanyl-tRNA synthetase (whose genetics is unknown). We found that the synthesis of the leucyl-tRNA synthetase in response to chloramphenicol, ethidium bromide and cycloheximide fully conformed to Barath and Küntzel's model. The production of phenylalanyl-tRNA synthetase activity in response to the inhibitors, however, suggests the involvement of either activation of extant enzyme or processing of an inactive precursor molecule as a large component of the increase in the specific activity of the enzyme after treatment with chloramphenicol.

The leucyl and phenylalanyl-tRNA synthetases are easily resolved and quantified by chromatography on standardised hydroxyapatite columns[5]. Figure 1 illustrates the resolution of the mitochondrial and cytoplasmic leucyl and phenylalanyl-tRNA synthetases on hydroxyapatite and the general observation that the amount of mitochondrial enzyme (first to elute from the column in both instances) increases as a function of chloramphenicol inhibition, whereas the amount of cytoplasmic enzyme decreases. Prolonged growth of *Neurospora* in the presence of 7 mg ml^{-1} chloramphenicol resulted repeatedly in a five- to sevenfold increase in specific activity of the mitochondrial leucyl-tRNA synthetase concomitant with a 50–70% reduction in the cytoplasmic enzyme. In the same circumstances the specific activity of the mitochondrial phenylalanyl-tRNA synthetase was found to increase four- to fivefold, whereas the activity of the cytoplasmic enzyme was reduced by approximately 30%. Similar increases in specific activity of the mitochondrial tRNA synthetases have been obtained

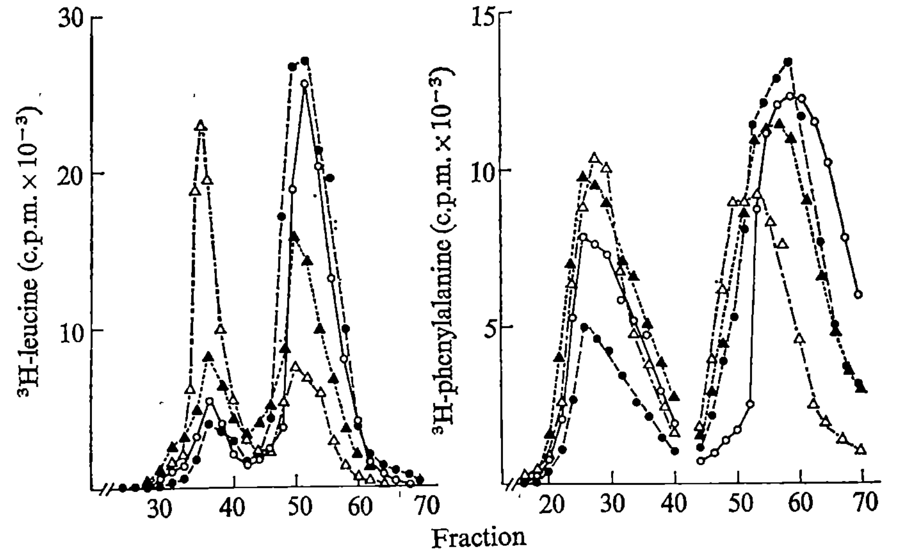

Fig. 1 Chromatographic resolution on hydroxyapatite of leucyl- (*a*) and phenylalanyl-tRNA synthetases (*b*) as a function of growth of *Neurospora* on chloramphenicol. The standard strain STD8A was grown for 20 h in the presence of 0 (●) and 2 mg ml^{-1} chloramphenicol (○) and for 35 h in the presence of 4 (▲) and 7 mg ml^{-1} chloramphenicol (△) at 34 °C with aeration in 1.0 l Vogel's synthetic medium. The inoculum was 40 ml of a 1.0 A_{550} conidial suspension. Extraction of mycelia, ammonium sulphate fractionation, hydroxyapatite chromatography and enzyme assays were carried out as described by Weeks and Gross[5]; 52 mg protein were applied to each of the columns of hydroxyapatite. The assay for leucyl-tRNA synthetase activity utilised *Escherichia coli* tRNA, which is charged by both the mitochondrial and cytoplasmic enzymes. The phenylalanine enzymes are quite specific; the mitochondrial enzyme charges only *Neurospora* mitochondrial or *E. coli* tRNA, whereas the cytoplasmic enzyme is very much more efficient in charging *Neurospora* cytoplasmic tRNA than *E. coli* tRNA[3,5]. For clarity, the data plotted for the phenylalanyl-tRNA synthetases are the charging of *E. coli* tRNA by the mitochondrial enzyme (fractions 16–40) and of *Neurospora* tRNA by the cytoplasmic enzyme (fractions 44–70).

after growth of *Neurospora* in the presence of 10 $\mu g\ ml^{-1}$ ethidium bromide. Since the mode of inhibitory action of ethidium bromide on mitochondrial protein synthesis is quite different from that of chloramphenicol—inhibiting preferentially mitochondrial DNA transcription[6,7]—the increase in the activities of the mitochondrial tRNA synthetases seems to be a consequence of inhibition of mitochondrial protein synthesis rather than of some specific effect of chloramphenicol.

To ensure that the increase in the mitochondrial leucyl-tRNA synthetase activity really represents an increase in the mitochondrial enzyme rather than some chloramphenicol-induced change in mobility of the cytoplasmic enzyme, the tRNA specificity, antibody sensitivity and electrophoretic mobility of the mitochondrial leucyl-tRNA synthetase from chloramphenicol-treated mycelia were examined and found to be identical to the enzyme obtained from untreated mycelia. In addition, 45208t (*leu-5*⁻), a mutant strain of *Neurospora* shown previously to produce little, if any, active mitochondrial leucyl-tRNA synthetase[3,5], failed to yield discernible quantities of the enzyme after growth in the presence of inhibiting concentrations of chloramphenicol. Furthermore, *leu-5*^{M}, a strain of *Neurospora* which produces an electrophoretic variant of the mitochondrial leucyl-tRNA synthetase (our unpublished results), responds to chloramphenicol inhibition by producing, in excess, only the *leu-5*^{M} mitochondrial enzyme.

In spite of severe inhibition of mitochondrial protein synthesis by chloramphenicol, both mitochondrial synthetases were found associated with the mitochondrial fraction. Indeed the specific activity of the two enzymes in the mitochondrial fraction was found to be elevated by a factor nearly equivalent to that of the whole cell extract. Thus, in spite of severe inhibition of mitochondrial protein synthesis by chloramphenicol[8] at concentrations that are supposed to damage intact mitochondrial[9], proteins find their way to, and become an integral part of, the organelle.

The increase in specific activity of mitochondrial leucyl and phenylalanyl-tRNA synthetases occurs quickly after transfer to medium containing chloramphenicol. The data in Table 1 indicate a two- to threefold increase in the specific activity of the leucyl-tRNA synthetase and a fivefold increase in the phenylalanine enzyme within 4 h. Neither enzyme is elevated during growth in the presence of cycloheximide alone, but the two enzymes respond very differently to combined treatment with chloramphenicol and cycloheximide. The specific activities of the mitochondrial and cytoplasmic leucyl-tRNA synthetases remain at 70–80% of the untreated control in the presence of chloramphenicol and cycloheximide at all concentrations used. Surprisingly, the increase in the specific activity of the mitochondrial phenylalanyl-tRNA synthetase in the

Table 1 Effect of cycloheximide on derepression of mitochondrial tRNA synthetases by chloramphenicol*

Chloramphenicol ($mg\ ml^{-1}$)	Cycloheximide ($\mu g\ ml^{-1}$)	Mycelia wet weight ($g\ l^{-1}$)	tRNA synthetase specific activities† (% control)		
			Mitochondrial		
			Leucyl	Phenylalanyl	Cytoplasmic leucyl
0	0	9.2	372 (100)	2,790 (100)	4.2×10^4 (100)
3	0	5.5	591 (159)	3,555 (127)	4.7×10^4 (112)
4	0	4.4	905 (243)	9,056 (325)	3.9×10^4 (93)
5	0	3.0	986 (265)	13,913 (499)	4.5×10^4 (107)
0	4	3.75	287 (77)	2,869 (103)	3.2×10^4 (76)
3	4	2.5	287 (77)	3,478 (125)	2.9×10^4 (69)
4	4	1.75	289 (78)	5,217 (186)	3.4×10^4 (81)
5	4	1.0	301 (81)	10,909 (391)	3.0×10^4 (71)

*Mycelia obtained after growth for 13 h in the absence of inhibitors, as described in Fig. 1, were divided in 3-g portions and resuspended in medium with varying amounts of chloramphenicol with or without cycloheximide. Mycelia were collected after 4.0 h growth without cycloheximide or 4.5 h growth with cycloheximide at 34 °C with aeration.

†The enzymes were resolved by hydroxyapatite chromatography and the active fractions pooled. The specific activities, determined on the basis of initial velocities, are in terms of c.p.m. ^{3}H-aminoacyl-tRNA formed per mg protein per min.

presence of chloramphenicol is not appreciably reduced by cycloheximide. The data suggest that the chloramphenicol-stimulated increase in activity of the mitochondrial leucyl-tRNA synthetase is dependent on cytoplasmic protein synthesis, whereas the increase in the activity of the phenylalanine enzyme is not.

The data of Table 1 also indicate that when both mitochondrial and cytoplasmic protein synthesis are inhibited, extensive degradation of the input mycelia occurs. This is demonstrated not only by a decrease in mycelial wet weight, but also qualitatively by the dark colour and rubbery consistency of the mycelia. Although we find this a troublesome consequence of simultaneously turning off mitochondrial and cytoplasmic protein synthesis, it does not significantly affect our conclusions, as the degradation seems random with respect to the enzymes studied. There is no correlation between weight loss and the specific activity of the leucine enzymes and there is reasonable correspondence between the increases observed in the specific activity of the mitochondrial phenylalanyl-tRNA synthetase in the presence of chloramphenicol with or without cycloheximide.

The pattern of synthesis of the mitochondrial leucyl-tRNA synthetase conforms to Barath and Küntzel's suggestion of the involvement of a controlling element synthesised in mitochondria that exercises control over the rate of transcription of nuclear genes specifying the structure of mitochondrial proteins. Credence to the model is added, at least as far as the mitochondrial leucyl-tRNA synthetase is concerned, by the fact that the structural gene has been located in the nucleus by conventional genetic analysis (our unpublished results), rather than by inference from patterns of inhibition of synthesis by specific antibiotics. In contrast to the above, the increase of mitochondrial phenylalanyl-tRNA synthetase activity after simultaneous inhibition of mitochondrial and cytoplasmic protein synthesis is incompatible with the model. Barring the existence of a protein-synthesising system insensitive to both cycloheximide and chloramphenicol, it must be assumed that the increase in activity is largely due to removal or decay of some specific enzyme inhibitor or to processing of a precursor of the mitochondrial phenylalanine-activating enzyme. (The precursor may be a component of the cytoplasmic enzyme. The cytoplasmic phenylalanyl-tRNA synthetase does not chromatograph as a homogeneous species[10] and, in addition to reduced specific activity, seems consistently to elute earlier when isolated from mycelia grown in the presence of chloramphenicol.) The extraordinary feature of the system is, however, that whatever the basis for the increase in specific activity of the phenylalanine-activating enzyme it is associated either directly or indirectly with disruption of mitochondrial protein synthesis. It seems likely that in addition to the genetic regulatory molecule(s) proposed by Barath and Küntzel, which is likely to be involved in regulating the synthesis of the mitochondrial leucine activating enzyme, some component of the mitochondrion is involved in the conversion of the precursor of the phenylalanine enzyme into the active enzyme with appropriate properties of localisation and substrate specificity.

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Evidence for post-transcriptional selection of viral mRNA in cells transformed by human adenovirus 12

CONTROL of gene expression occurs mainly at the transcriptional level in prokaryotic cells[1]. In eukaryotic cells, the sites of transcription and translation are physically separated by the nuclear membrane, giving rise to the possibility of regulation by post-transcriptional mechanisms. Control could be exercised at several stages of mRNA biogenesis: methylation, polyadenylation, cleavage, or transport across the nuclear membrane[2,3].

The transcription of DNA tumour virus genes provides a useful model for analysing post-transcriptional regulation in eukaryotic cells. The expression of integrated virus genes in transformed cells is thought to be regulated by host cell mechanisms. There is evidence to suggest that in adenovirus-transformed cells viral mRNA formation shares important features with cell mRNA formation[4–7]. Viral DNA probes of very high specific radioactivity can be prepared to detect both stable cytoplasmic mRNA as well as short lived nuclear viral transcripts. Using such probes, we show here that only a fraction of nuclear viral RNA sequences are exported to the cytoplasm in hamster cells transformed by highly oncogenic human adenovirus 12 (Ad 12).

The Ad 12-transformed hamster cell line, HE C19, contains multiple copies of all or nearly all of the viral genome in an integrated state and different portions of the viral genome are present in equimolar quantities[8]. These cells, therefore, provide a model to study the expression of a specific integrated multigenic sequence. In this study, we determined the fraction of the Ad 12 genome expressed as viral RNA sequences in the nucleus and the cytoplasm of HE C19 cells using *in vitro* labelled Ad 12 DNA probes of very high specific activity. Nuclear, cytoplasmic, and polyribosomal RNA were isolated from HE C19 cells, previously shown to transcribe relatively large quantities of virus-specific RNA[9]. Cellular RNA in vast excess (2–10 mg ml^{-1}) was annealed with Ad 12 ^{32}P-DNA (2×10^8–4×10^8 c.p.m. μg^{-1}) labelled *in vitro* by the "nick translation" reaction of *E. coli* DNA polymerase 1 (refs 8 and 10). The high ratios of RNA to DNA (Table 1) enabled the detection of viral RNA species present in low abundance. These "scarce" RNA sequences are not detected by probes of lower specific activity (10^6–10^7 c.p.m. μg^{-1}) such as those prepared by *in vivo* labelling (not shown). As shown in Fig. 1, cytoplasmic RNA saturated 10–11% of viral DNA after 6 h of annealing; this value was not increased after 24 h. We conclude that 10–11% of the duplex viral DNA genome representing 20–22% of its asymmetric coding capacity gives rise to cytoplasmic RNA. Similar experiments with RNA isolated from two polyribosome preparations gave identical results —10–12% hybridisation saturation (Table 1). At least 93% of the viral DNA sequences are integrated into the cellular genome[8]. Thus, more than 70% of the potential viral genetic information does not give rise to mRNA.

To determine whether viral RNA sequences are present in the nucleus that are not exported to the cytoplasm, nuclear RNA (2 mg ml^{-1}) was annealed with Ad 12 ^{32}P-

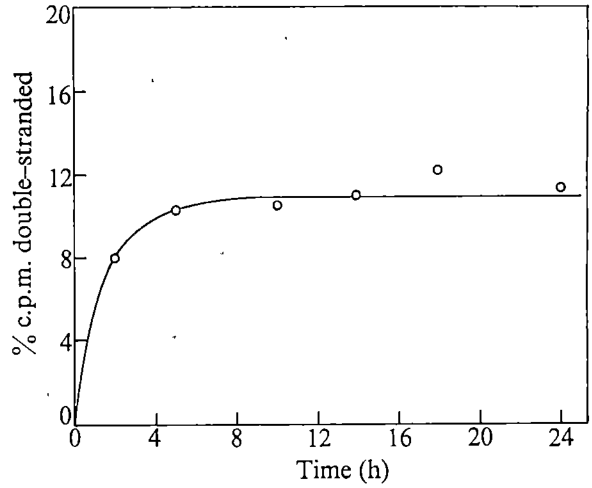

Fig. 1 Hybridisation of *in vitro* Ad 12 ^{32}P-DNA with HE C19 cytoplasmic RNA. Ad 12 viral DNA was prepared as described[11,12] and labelled with ^{32}P-deoxyribonucleoside triphosphates to specific activities of 2×10^8–4×10^8 c.p.m. μg^{-1} by the nick translation reaction of *E. coli* DNA polymerase 1 (ref. 10) essentially as described by Paul Berg *et al.* (personal communication), scaled down to 4 μl reaction mixture (J K.M., K. Brackmann, M.R.G., and M.G., unpublished). Heat denatured Ad 12 ^{32}P-DNA (800–1,000 c.p.m.) was annealed at 68 °C with HE C19 cytoplasmic RNA (10 mg ml^{-1}) in 5 μl of 0.72 M NaCl, 10 mM PIPES (*p*H 6.7), 1 mM EDTA, and 0.05% SDS in sealed siliconised capillary tubes (Fisher). Tubes were removed at the indicated times, cooled to 4 °C, and transferred to a solution containing 30 mM sodium acetate (*p*H 4.5), 50 mM NaCl, 1 mM $ZnSO_4$, 5% glycerol, and 40 μg denatured calf thymus DNA. Samples were split equally into two test tubes and S1 nuclease was added to one of the tubes. The reaction mixtures were incubated for 1 h at 50 °C and the fraction of duplex DNA determined by measurement of trichloroacetic-acid-precipitable radioactivity. The extent of self-annealing of the probe for all time points was determined in reactions substituting 10 mg ml^{-1} of yeast tRNA for HE C19 RNA. The self-annealing background (less than 8% after 72 h) was subtracted from each value. The average of duplicate values are plotted in the figure. To prepare HE C19 RNA, cells were collected, washed twice with phosphate buffered saline, and resuspended in 10 volumes of ice-cold isotonic high *p*H (Iso-hi-*p*H) buffer[13] containing 40 μg ml^{-1} of dextran sulphate. Nonidet P-40 (0.5%) (Shell) was added to lyse cells All steps were performed at 4 °C. Nuclei were collected by centrifugation at 800*g*. The cytoplasmic supernatant was precipitated with 2.5 volumes of 95% ethanol and 0.3 M NaCl at −20 °C overnight. The precipitate was collected by centrifugation resuspended in 10 ml of 0.1 M Tris-HCl (*p*H 9.0) and 0.5% SDS, and extracted three times with equal volumes of water-saturated phenol. The phenol phase was extracted with an equal volume of buffer, the aqueous phases combined, and alcohol precipitated. The pellet was resuspended in 5–10 ml of 0.1 M NaCl, 1 mM $MgCl_2$, 0.01 M Tris-HCl (*p*H 7.4), and incubated with 100 μl ml^{-1} of iodoacetate-treated[14], electrophoretically purified pancreatic DNase (Worthington) for 1 h at 37 °C, followed by three extractions with equal volumes of water-saturated phenol. RNA was precipitated, DNase treated, phenol extracted, and again precipitated. RNA was dissolved in 1 ml of 0.01 × SSC and passed through a Sephadex G-100 column to remove DNA fragments. The peak of RNA was alcohol precipitated and resuspended in a small volume of 0.01 × SSC. Polyribosomes were prepared from the cytoplasmic supernatant after removal of mitochondria by centrifugation at 12,000*g* for 10 min in a Sorvall at 4 °C. The supernatant was layered on to a discontinuous sucrose gradient containing 2 ml of 2 M sucrose and 2 ml of 1.28 M sucrose made up in Iso-hi-*p*H buffer plus 100 μg ml^{-1} dextran sulphate. Polyribosomes were collected by centrifugation at 45*K* in a Spinco Ti50 rotor at 4 °C for 3 h or overnight. The polyribosome pellet was suspended in 10 ml of 0.1 M Tris-HCl (*p*H 9.0) and 0.5% SDS, and RNA was isolated as described above for total cytoplasm. The absence of viral DNA was confirmed by alkaline hydrolysis (0.2 N KOH, 100 °C, 15 min) of a small portion of the RNA preparations followed by hybridisation to *in vitro* labelled Ad 12 ^{32}P-DNA; in all cases less than 1% hybridisation above self-annealing background was observed after 48 h. S1 nuclease was prepared from *Aspergillus oryzae* α-amylase. The α-amylase was stirred for 1 h at 4 °C in 20 mM sodium acetate (*p*H 4.5), 0.1 mM $ZnSO_4$, 50 mM NaCl, and 5% glycerol, and the precipitate removed by centrifugation. The supernatant was adjusted to *p*H 5.0, heated to 70 °C with vigorous stirring, and quick-chilled in ice. Following removal of the precipitate by centrifugation, the enzyme was purified by DEAE cellulose chromatography as described[15]. The enzyme was stored in 50% glycerol at −20 °C.

DNA. As shown in Fig. 2, 24% of the viral DNA probe hybridised by 24 h, and 38% by 84 h. Since saturation was not reached at this time, 38% is a minimal estimate of the viral sequence content of nuclear RNA. Analysis of a different nuclear RNA preparation showed 31% hybridisation at 24 h (Table 1). Several factors may account for the inability of nuclear RNA to saturate the viral DNA probe: (1) some viral RNA species may be present in very small amounts; (2) RNA is degraded during long hybridisation periods; (3) RNA–RNA hybridisation of putative symmetrical RNA transcripts may compete with the RNA–DNA hybridisation reaction.

Highly radioactive viral DNA probes could detect small amounts of viral DNA that may contaminate RNA preparations. The following precautions eliminate this possibility. Nuclear and cytoplasmic RNAs were digested two or three times with pancreatic DNase which in our hands destroys all viral DNA even in RNA preparations from productively infected cells; the latter contain 10^5 times more viral DNA than do transformed cells. Diphenylamine analysis on nuclear RNA (800 μg) detected less than one-tenth the amount of viral DNA necessary to drive the hybridisation reaction. Alkaline hydrolysis of nuclear RNA eliminated hybridisation to viral DNA (see legend to Fig. 1).

This is the first demonstration to our knowledge that viral RNA sequences are present in the nucleus of a transformed cell which are not transported to the cytoplasm. It is possible that the non-mRNA sequences in the nucleus arise through errors in transcription, and that the expression of integrated Ad 12 genes is primarily regulated at the transcriptional level. Even so, our data suggest that strong post-transcriptional controls must operate to transport selectively mRNA sequences to the cytoplasm, and to restrict non-mRNA sequences to the nucleus. This is analogous to mRNA biogenesis in uninfected eukaryotic cells in which most nuclear RNA sequences do not reach the cytoplasm[3,16,17]. Our data provide preliminary evidence for post-transcriptional regulation of gene expression as distinguished from RNA processing since nearly all the Ad 12 genome specifies mRNA, that is late after productive

Table 1 Hybridisation of HE C19 with *in vitro* labelled Ad 12 ^{32}P-DNA

	HE C19 RNA	Ad 12 ^{32}P-DNA	RNA/DNA	Longest hybridisation time (h)	Largest R_0t	% DNA hybridisation
1	Cytoplasm (prep 1)	Probe 1 (2×10^8 c.p m. μg)	1.2×10^7	24	2,880	11
2	Polysomal (prep 1)	Probe 1 (2×10^8 c.p.m. μg)	7.5×10^6	48	3,458	12
3	Polysomal (prep 2)	Probe 2 (3.5×10^8 c.p.m. μg)	1.3×10^7	25	1,800	10
4	Nuclear (prep 1)	Probe 1 (2×10^8 c.p.m. μg)	2.5×10^6	84	2,016	38
5	Nuclear (prep 2)	Probe 3 (2.5×10^8 c.p.m. μg)	3.1×10^6	24	576	31

RNA was prepared as described in the legends to Figs. 1 and 2.

Cytoplasmic RNA (10 mg ml^{-1}), polysomal RNA (6 mg ml^{-1}), or nuclear RNA (2 mg ml^{-1}) were annealed using standard hybridisation conditions described in Fig. 1. Reactions were stopped at the specified times and assayed for ^{32}P-DNA hybridised. The value for the last time points are given in this table.

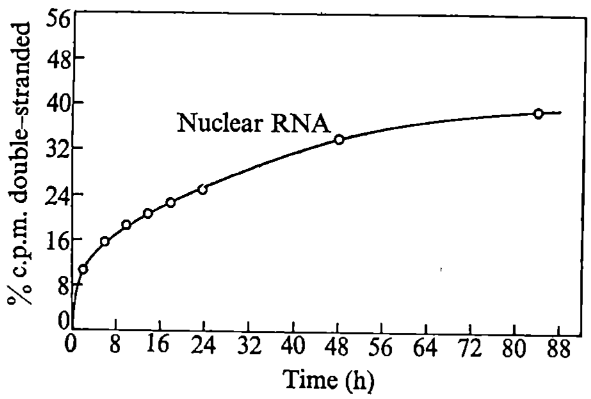

Fig. 2 Hybridisation of *in vitro* Ad 12 ^{32}P-DNA with HE C19 nuclear RNA. Nuclei were collected as described in the legend to Fig. 1 and washed twice with 10 volumes of Iso-hi-*p*H buffer plus one-tenth volume of detergent mix (2 parts 10% Tween-40, 1 part 10% deoxycholate). The pellet was resuspended in 10 volumes of 0.05 M sodium acetate, 0.01 M EDTA, *p*H 5.1 and 0.5% SDS, dispersed with a Dounce homogeniser, and extracted with an equal volume of water-saturated phenol plus one half volume of chloroform–isoamyl alcohol (24:1) at 68 °C for 3 min. The phenol phase was extracted with one half volume of Iso-hi-*p*H buffer at 4 °C. The aqueous phases were combined and extracted with an equal volume of water-saturated phenol and one half volume of chloroform–isoamyl alcohol at 4 °C. RNA was precipitated, dissolved in 10 ml of 0.1 M NaCl, 1 mM $MgCl_2$, 0.01 Tris-HCl (*p*H 7.4), and DNase treated, phenol extracted, and precipitated. The DNase treatment was repeated twice and the RNA further purified as described. Alkaline hydrolysis was performed on all RNA preparations; less than 1% hybridisation above self-annealing background, was observed after 48 h. Hybridisation was performed as described in the legend to Fig. 1 except that 2 mg ml^{-1} of nuclear RNA was used instead of 10 mg ml^{-1} of cytoplasmic RNA.

infection, polyribosomal RNA saturates 48–49% of Ad 12 ^{32}P-DNA, representing over 95% of the asymmetrical coding capacity of the viral genome (unpublished data). By contrast, most of the eukaryotic genome does not code for mRNA[3,17,18]. Our data do not, however, exclude the formal but unlikely possibility that the sequences we see in the nucleus but not in the cytoplasm represent anti-mRNA from about 60% of the viral genome that never exit even during lytic infection.

Our data provide no direct evidence that primary RNA transcripts are larger than viral mRNA molecules and do not distinguish between two possibilities: that the large primary RNA transcripts are cleaved and processed to smaller ones that are exported to the cytoplasm; or that discrete smaller RNA transcripts are generated, but only some of these are transported to the cytoplasm. Recent studies have suggested that during late stages of productive Ad 2 infection, primary transcripts are large polycistronic molecules derived from more than half of the viral genome, and that these exhibit a precursor–product relationship with smaller cytoplasmic mRNA[19]. It is not known, however, whether the transcription of the Ad genome late in productive infection resembles transcription of host cell DNA and integrated viral genes. Further studies with the Ad 12-transformed cell system provide the opportunity to use highly radioactive restriction fragments to study the "discarded" nuclear sequences. An analysis of the properties of the nuclear RNA molecules containing these sequences will be most useful in evaluating the significance of post-transcriptional mechanisms in the primary route of eukaryotic mRNA biogenesis.

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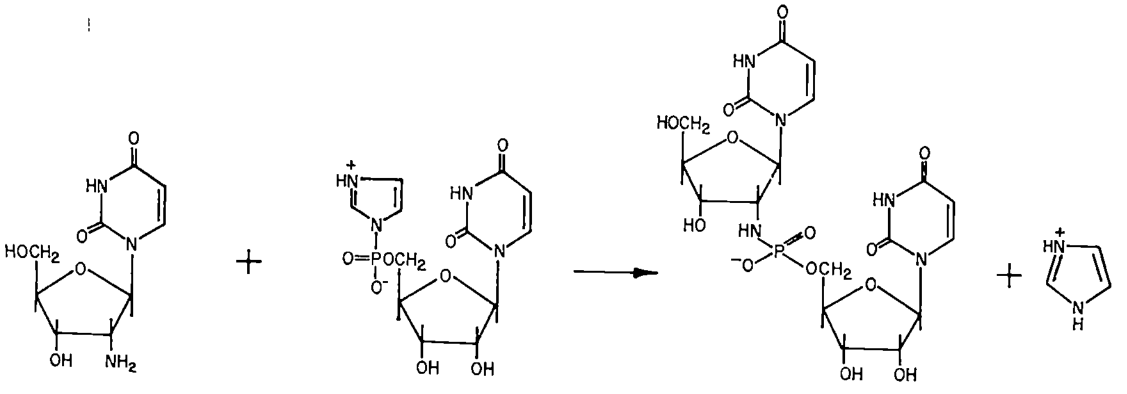
Template-directed synthesis of high molecular weight polynucleotide analogues

A GREAT deal is known about the structures of nucleic acids and the mechanisms involved in nucleic acid replication. This relatively new knowledge has led a number of authors to speculate that other linear heteropolymers might be able to serve as carriers of genetic information. A number of polynucleotide analogues have been synthesised by chemical or enzymatic methods[1-7] and one of them has been used as a template for RNA polymerase[4]. Two studies, in particular, have some relationship to the present work. 3′-5′-Linked polynucleotides derived from 2′-amino-2′-deoxynucleoside 5′-phosphates have been synthesised enzymatically[7]. Letsinger *et al.*[5,6] reported the chemical and enzymatic formation of polydeoxyribonucleotides containing N$^{5'}$–O$^{3'}$-phosphoramidate linkages. Here we discuss nucleic acid analogues derived from 2′-amino-2′-deoxynucleotides that we believe may be capable of non-enzymatic replication, and describe some experimental results which, although they are far from establishing the principle of self-replication, do show that it is possible to synthesise fairly long oligomers on templates in aqueous solution.

Polynucleotides have been shown to act as templates for the non-enzymatic condensation of complementary mononucleotides and oligonucleotides[8]. Our own work indicates that, whenever ribonucleotide derivatives are used in template-directed reactions, 2′–5′ linkages are formed in excess over 3′–5′ linkages. Furthermore, we have found it difficult to achieve an efficiency of condensation greatly in excess of 50% (ref. 9). These observations suggest that a much more effective template-directed reaction could be achieved by using, instead of ribonucleotides, derivatives in which the 2′-hydroxy group is replaced by a more nucleophilic group, for example an SH, NH_2 or NHOR group. So far our experimental work has been restricted to the 2′-amino-2′-deoxynucleotides.

We established first that 2′-amino-2′-deoxyuridine (U_{NH_2})[10] condenses effectively with uridine 5′-phosphorimidazolide (ImpU) or adenosine 5′-phosphorimidazolide (ImpA) in



(U_NpU)

Fig. 1 Condensation of U_{NH_2} with ImpU (or ImpA) to give dinucleoside phosphates.

aqueous solution to give dinucleoside phosphates as shown in Fig. 1. High voltage electrophoresis of the product obtained with ImpA establishes that it has zero charge at *p*H 2.7. This is consistent with a 2′–5′-phosphoramidate structure and incompatible with a 3′–5′-linked phosphodiester carrying a free 2′-amino group, since a compound with the latter structure would carry a positive net charge at *p*H 2.7. The presence of a phosphoramide bond in our product is further indicated by its hydrolysis in 0.1 M formic acid at room temperature[5,6] ($t_{1/2}$ 70–80 h). The hydrolysis of adenylyl-[5′→2′]-2′-amino-2′-deoxyuridine (U_NpA) at various *p*Hs will be discussed in another paper[11].

In aqueous imidazole buffer at *p*H 7.5 and 0 °C, U_{NH_2} (ref. 10) reacts with ImpA or ImpU 50–100 times faster than does uridine (U). Yields of U_NpA of about 80% were obtained from solutions of 0.1 M in U_{NH_2} and 0.01 M ImpA. Similar yields of uridylyl-[5′→2′]-2′-amino-2′-deoxyuridine (U_NpU) were obtained in the reaction of U_{NH_2} with ImpU. In identical conditions, uridine reacted with ImpA and ImpU to give at most a few per cent of dinucleoside monophosphate[11].

A preliminary experiment with 2′-amino-2′-deoxyuridine 5′-phosphorimidazolide ($ImpU_{NH_2}$) showed that the enhanced reactivity of the amino group also facilitates the synthesis of oligomers. We prepared a concentrated solution of $ImpU_{NH_2}$ (about 0.8 M) in 2.0 M imidazole buffer. After keeping the reaction mixture for 28 days at 0 °C we obtained a 62% yield of a complex mixture of oligomers of 2′-amino-2′-deoxyuridine 5′-phosphate (pU_{NH_2}) including 11.9% moving slower than the

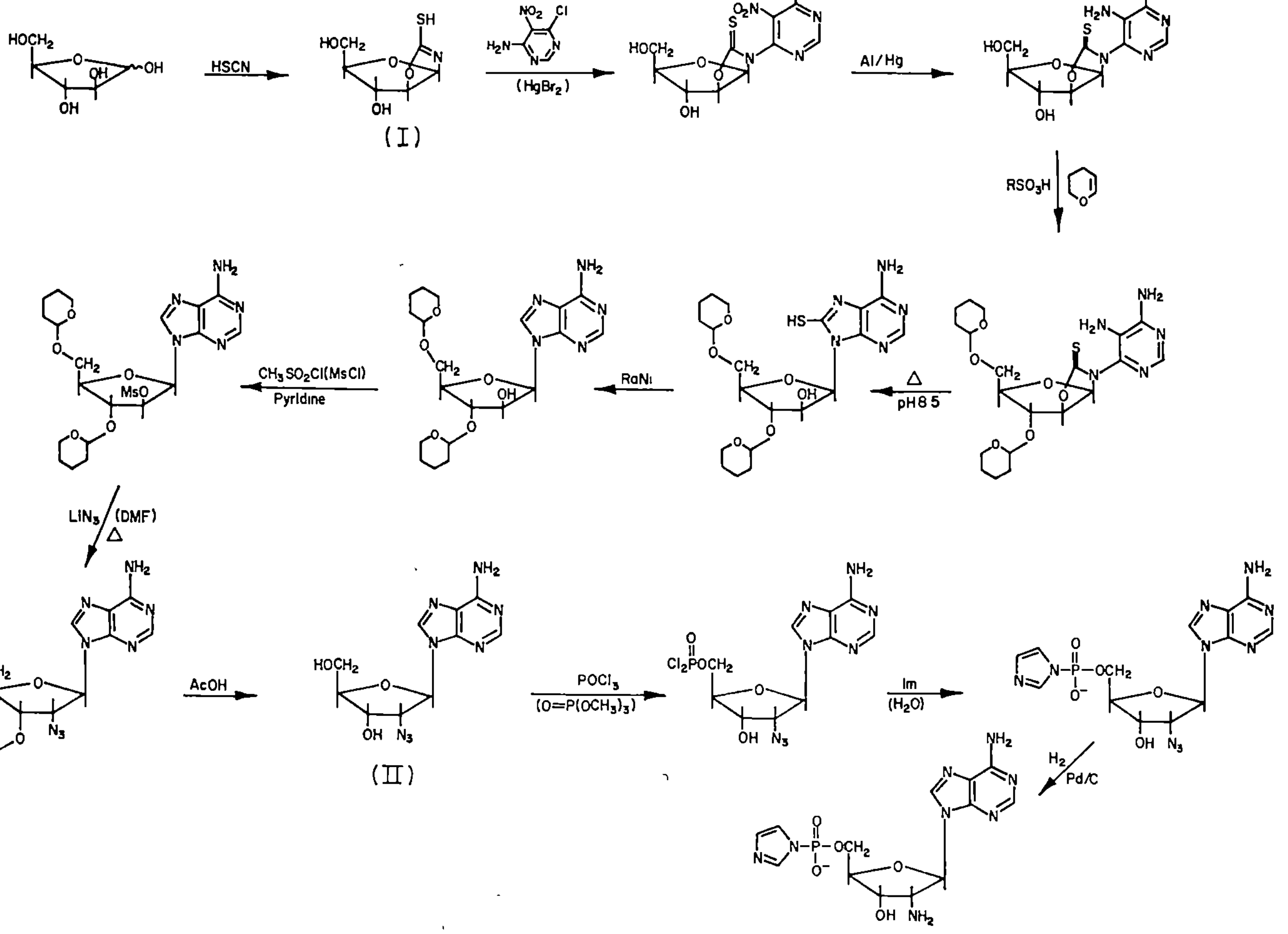


Fig. 2 Scheme for synthesis of $ImpA_{NH_2}$.

pentauridylate (Up)$_5$ on chromatography in n-propanol–ammonia–water. In the same conditions ImpU gave very little high molecular weight material.

The previous demonstration that template-directed reactions are much more efficient than reactions in free solution, and yield 2′–5′-linked products, together with the present finding that a 2′-amino group is 50–100 times more reactive than a 2′-hydroxy group in the absence of a template, led us to synthesise 2′-amino-2′-deoxyadenosine 5′-phosphorimidazolide (ImpA$_{\mathrm{NH_2}}$). This enabled us to study the reactivity of a 2′-amino group in a template-directed condensation on polyuridylic acid (poly (U)).

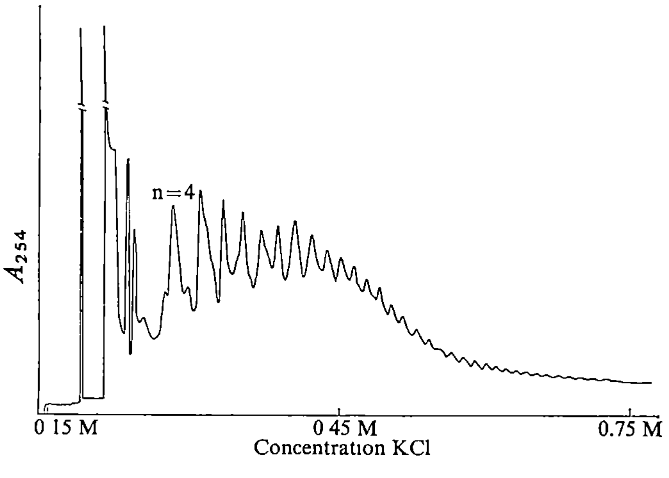
We prepared ImpA$_{\mathrm{NH_2}}$ by the route outlined in Fig. 2. The key compound, 2′-azido-2′-deoxyadenosine (II) was obtained in an overall yield of about 8% based on arabinofurano-thionoxazolidine (I). It melted at 215–216 °C (w. dec.). The ultraviolet spectrum of II ($\lambda_{\max}$ at *p*H 1.0, 257 nm (ε = 14,900); at *p*H 6.4, 259 nm (ε = 15,000); at *p*H 12.0, 258 nm (ε = 14,900)) and the nuclear magnetic resonance spectrum were consistent with the proposed structure. Furthermore, II was reduced catalytically to 2′-amino-2′-deoxyadenosine, which had a specific optical rotation of $[\alpha]_D^{22} = 65.1°$ (0.50%, methanol). The reported value for $[\alpha]_D^{22}$ is $-66\pm2°$ (ref. 12). We are indebted to Dr R. Ranganathan (ref. 13) for drawing to our attention his novel synthesis of ara A and his preliminary work on the protection of the hydroxyl groups of (I) with tetrahydropyranyl groups.

A solution containing poly(U) (0.05 M), ImpA$_{\mathrm{NH_2}}$ (0.025 M), $MgCl_2$ (0.075 M) and NaCl (0.2 M) was maintained at 0 °C and *p*H 7.5 for 16 d. The reaction mixture was treated with pancreatic ribonuclease to degrade the poly(U); the resulting solution was loaded on a RPC-5 column and eluted with a KCl gradient. In Fig. 3 we show a record of the ultraviolet absorption (254 mμ) of the eluate.

In independent experiments we found that 2′–5′-linked oligomers (pA$_{\mathrm{N}}$)$_n$ of 2′-amino-2′-deoxyadenosine 5′-phosphate (pA$_{\mathrm{NH_2}}$) with n = 1–6 move slightly behind the corresponding 3′–5′-linked oligomers of adenosine 3′-phosphate on paper chromatography in *n*-propanol–concentrated ammonia–water (55 : 10 : 35). We desalted the materials corresponding to early peaks from the column, and subjected them to chromatography in that system. In this way we were able to estimate the chain length of the early peaks. This enabled us to conclude that the last detectable peak from the column corresponded to an oligomer about 35 units long. The total yield of material in dimer and oligomers amounted to about 87% of the reacted ImpA$_{\mathrm{NH_2}}$.

Control experiments in which the poly(U) template was omitted, showed that substantial amounts of dimer and some trimer are formed, but only very small amounts of higher oligomers. Thus the presence of a poly(U) template facilitates the synthesis of (pA$_{\mathrm{N}}$)$_n$ up to the 35-mer in conditions of dilution in which no high molecular weight material is detected in the absence of a template.

Fig. 3 Products from poly(U)-directed condensation of ImpA$_{\mathrm{NH_2}}$. The trace is the record of the ultraviolet absorbance at 254 nm of the eluate from a RPC-5 column.



The non-catalysed condensation reactions of activated nucleotides are very inefficient in the absence of templates, and only moderately efficient in their presence. Our experiments show that the corresponding reactions of activated 2′-amino-2′-deoxynucleotides are much more efficient. In concentrated aqueous solution complex mixtures of moderately long oligomers are formed, whereas efficient template-directed condensations occur on suitable polymers in fairly dilute solution to give (pA$_{\mathrm{N}}$)$_n$. This naturally raises the question of the relevance of the analogues to the origins of life. Could a replicating system have evolved from activated aminonucleotides, and only later have been replaced by the present system of nucleic acids?

It is easy to formulate schemes for the synthesis of 2′-amino-2′-deoxyribose from ribose or ribulose and ammonia (reviewed in ref. 14), but we have been unable to obtain appreciable yields of the amino sugars in this way. Furthermore, our attempts to obtain aminonucleosides from amino sugars in prebiotic conditions were unpromising. Since we did not make extended studies of the reactions we cannot exclude the possibility that a prebiotic synthesis of amino nucleotides is possible. Until such a synthesis is reported, however, we will concentrate on 'non-prebiotic' studies of the reactions of aminonucleotides.

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Homology relationships among the small blue proteins

COMPARATIVELY few blue proteins, all containing copper, are found in nature. Isolated from organisms all along the evolutionary scale they are[1] the 'blue oxidases'—laccase and ascorbate oxidase of plant origin and ceruloplasmin (ferroxidase) from mammalian serum (molecular weights 60,000–140,000, 4–8 Cu mol^{-1})—and the 'small blue proteins'—azurins from bacteria, plastocyanins from photosynthetic cells and others like stellacyanin and umecyanin, found in higher plants (molecular weights, carbohydrate excluded, 11,000–14,000, 1 Cu mol^{-1}). The small blue proteins may all function as electron carriers although a precise role is known only for the plastocyanins.

The so-called "blue" or "type 1" cupric copper in these proteins' has been well characterised by spectroscopic methods[2], and is always found to have the same general properties, not mimicked by any low molecular weight complexes. These are a typical optical spectrum with $\lambda_{\max}$

Azurins

```
   1        10        20        30        40        50        60        70        80        90        100       110       120       130
1  AECSVDIQGNDQMQFNTNAITVDKSCKQFTVNLSHPGNLPKNVMGHNWVLSTAADMQGVVTDGMASGLDKDYLKPDDSRVIAHTKLIGSGEKDSVTFDVSKLKEGEQYMFFCTFPGHSALMKGTLTLK-
2   E SVDIQGN Q QFSTNAITVD S  T  VN S P SLPKNV    W LTTAA MQGVVT  MAA LDKN V DG T VI   KII S  K  V F  S  KAGDA AF  S    SAM K TLT K-
3   E KTTIDST Q SFNTKAIEID S  T  VE S T SLPKNV    L ISKQA MQPIAT  LSA IDKN L EG T VI   KVI A  K  L I  S  NAAEK GF  S    ISM K TVT K-
4   E KVTVDST Q SFDTKAIEID S  T  VD K S NLPKNV    W LTTQA MQPVAT  MAA IDKN L EG T II   KII A  T  V F  S  KADGK MF  S    IAM K TVT K-
5   E KVDVDST Q SFNTKEITID S  T  VN T S SLPKNV    W LSKSA MAGIAT  MAA IDKD L PG S VI   KII S  K  V F  S  TAGES EF  S    NSM K AVV K-
6   E SVDIAGT Q QFDKKAIEVS S  Q  VN K T KLPRNV    W LTKTA MQAVEK  IAA LDNQ L AG T VL   KVL G  S  V F  A  AAGDD TF  S    GAL K TLK VD
7   Q EATIESN A QYDLKEMVVD S  Q  VH K V KMAKAV    W LTKEA KEGVAT  MNA LAQD V AG T VI   KVI G  S  V F  S  TPGEA AY  S    WAM K TLK SN
8   - DVSIEGN S QFNTKSIVVD T  E  IN K T KLPKAA    V VSKKS ESAVAT  MKA LNND V AG E VI   SVI G  T  V F  S  KEGED AF  S    WSI T EIK GS
9   E SVDIAGN G QFDKKEITVS S  Q  VN K P KLAKNV    W LTKQA MQGAVN  MAA LDNN V KD A VI   KVI G  T  V F  S  AAGED AY  S    FAL K VLK VD
```

Plastocyanins

```
   1        10        20        30        40        50        60        70        80        90        100
1  --LDVLLGGDDGSLAFIPGNFSVSAGEKITFKNNAGFPHNVVFDEDEIPAGVDASKISMAEEDLLNAAGETYSVTL--SE-KGTYTFYCAPHQGAGMVGKVTVN-
2  --LEVL  SGD S V V SEFSVPS EKIVFK  AGF   V   EDEI AGVDAVKI MPEEEL NAP ETYVVTL--DT-K T SFY S  Q    V KV  N-
3  --VEVL  ASD G A V NSFEVSA DTIVFK  AGF   V   EDEI SGVDAAKI MPEEDL NAP ETYSVKL--DA-K T KFY S  Q    V QV  N-
4  --VEIL  GED S A V GNFSVPS EKITFK  AGF   V   EDEV SGVDSAKI MSEDDL NAP ETYSVTL--TE-S T KFY S  Q    V KV  N-
5  --IEVL  GDD S A I NDFSVAA EKIVFK  AGF   V   EDEI SGVDAGKI MNEEDL NAP EVYKVNL--TE-K S SFY S  Q    V KV  N-
6  --VEVL  GGD S A L GDFSVAS EEIVFK  AGF   V   EDEI SGVDAAKI MSEEDL NAP ETYKVTL--TE-K T KFY S  Q    V KV  N-
7  -DVTVK  ADS A V E SSVTIKA ETVTWV  AGF   I   EDEV SAGNAEAL --HEDY NAP ESYSAKF--DT-A T GYF E  Q    K TI  Q-
8  ETYTVK  SDK L V E AKLTIKP DTVEFL  KVP   V   AALN AKSADLAK LSHKQL MSP QSTSTTFPADAPA E TFY E  R    V KI  AG
```

Fig. 1 Amino acid sequences for the 9 azurins and 8 plastocyanins considered, as collected from the literature. Amino acid residues are represented by their one-letter symbols[6]. Blank indicates that the residue is the same as in the first row and hyphen indicates a deletion. In the azurins Cys 3 and Cys 26 are connected by a disulphide bridge. Sources and references are as follows: Azurins (ref. 3 and R. P. Ambler, personal communication), 1, *Pseudomonas aeruginosa* P6009; 2, *P. denitrificans* NCIB 9496; 3, *P. fluorescens* B-93; 4, *P. fluorescens* C-18; 5, *P. fluorescens* D-35; 6, *Bordetella bronchiseptica* NCTC 8344; 7, *Alcaligenes denitrificans* NCTC 8582; 8, *A. faecalis* NCIB 8156; 9, *Alcaligenes* spp. Plastocyanins, 1, Potato (*Solanum tuberosum*)[16]; 2, French bean (*Phaseolus vulgaris*)[17]; 3, Broad bean (*Vicia faba*)[18]; 4, Elder (*Sambucus niger*)[19]; 5, Vegetable marrow (*Cucurbita pepo*)[20]; 6, Spinach (*Spinacea oleracea*)[21]; 7, Green algae (*Chlorella fusca*)[4]; 8, Blue-green bacteria (*Anabaena variabilis*)[22].

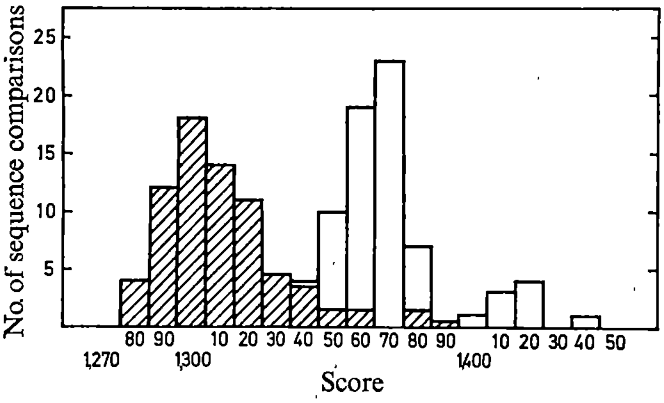

Fig. 2 Distribution of scores for best alignment of azurins with plastocyanins (72 pairs) before (open) and after (cross hatched) random permutations. Two random permutations were done for each pair, but the frequency numbers above are reduced by half to make the two distributions comparable. All bars, also overlapping, start at the bottom of the diagram.

at 610 nm having a molar extinction coefficient around 4,000, a very small hyperfine constant in the electron spin resonance spectrum and a high, albeit varying, redox potential. This indicates that the copper environment is very similar in all of them. This situation—if true—can be the result of either convergent evolution, where similar requirements have promoted the evolution of the same structure from different origins, or divergent evolution, where several or all of these proteins have a common ancestor. We have attempted to discriminate between these possibilities for the small blue proteins by comparing amino acid sequences that have recently become available for azurins and plastocyanins and, to some extent, for stellacyanin and umecyanin. The results suggest that all the small blue proteins are monophyletic in origin and indicate some of the conserved amino acid residues as potential copper ligands. The apparent similarity between the azurin and plastocyanin primary structure around the single cysteine residue has been noted before[3,4].

The nine pseudomonad azurin sequences plus six higher plant, one blue-green alga and one blue-green bacterium (prokaryote) plastocyanin sequences considered are shown in Fig. 1. Teh azurins have 128–129 amino acids, the plastocyanins 98–104 amino acids. The single disulphide bridge in the azurins is not found in the plastocyanins. Alignment of sequences and score for best alignment was obtained with a computer procedure based on the Needleman and Wünsch[5] algorithm. Weights for amino acid pairs, intended for detection of distant relationships, were taken from ref. 6.

Scores were calculated for all 72 possible pairs of azurins with plastocyanins plus two random permutations for each of those pairs. The distributions obtained are shown in Fig. 2; means and standard deviations are reported in Table 1. The distributions for the experimental and random sequences are different at a level of significance of 10^{-80} (assuming Gaussian shape). The nine values in both populations that deviate most from their respective means involve the prokaryotic plastocyanin comparisons, for

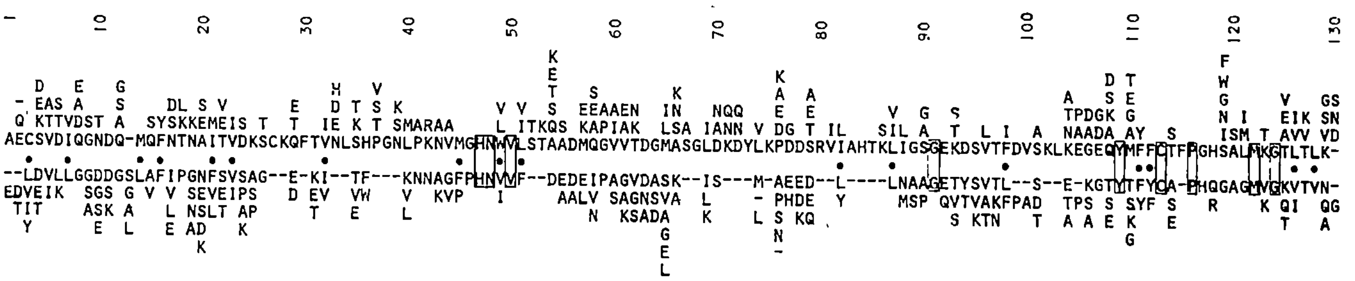
1 10 20 30 40 50 60 70 80 90 100 110 120 130
K F
E K D T W
D E G H V T S K A A A S E G V GS
- EAS A S DL S V E D T S K V V S EEAAEN IN NQQ E E V G S TPDGK G N I EIK SN
Q KTTVDST A SYSKKEMEIS T T IE K T SMARAA L ITKQS KAPIAK LSA IANN V DG T IL SIL A T L I A NAADA AY S ISM T AVV VD
AECSVDIQGNDQ-MQFNTNAITVDKSCKQFTVNLSHPGNLPKNVMGHNWVLSTAADMQGVVTDGMASGLDKDYLKPDDSRVIAHTKLIGSGEKDSVTFDVSKLKEGEQYMFFCTFPGHSALMKGTLTLK-
--LDVLLGGDDGSLAFIPGNFSVSAG--E-KI--TF---KNNAGFPHNVVF--DEDEIPAGVDASK--IS---M-AEED--L----LNAAGETYSVTL--S--E-KGTYTFYCA-PHQGAGMVGKVTVN-
EDVEIK SGS G V V SEVEIPS D EV VW V KVP I AALV SAGNSVA L - PHDE Y MSP QVTVAKFPAD TPS S SYF S R K QI QG
TIT ASK A L NSLT AP T E L N KSADA K L S KQ S KTN T A A E K E T A
Y E L E AD K G N G
K E -
L

Fig. 3 Alignment of a composite azurin (above) and composite plastocyanin (below) amino acid sequence. Each position in the composite sequence contains all residues observed there in the individual sequences (Fig. 1). Unvaried residues are shown in boxes whereas conserved aromatic or hydrophobic character is marked with dots.

which no correction was made for its five extra amino acids.

The deletion patterns in the shorter plastocyanins in the above alignments varied considerably although about 80% of the deletions were found in the middle third. A specific deletion pattern was obtained by comparing a composite azurin with a composite plastocyanin sequence. In these each position contained, regardless of their original frequencies of occurrence, one of each amino acid residue observed there. Weights for pairs were taken as the arithmetic mean of the weights for all possible pairs. The composite sequences and their alignment are shown in Fig. 3. Out of the 130 positions, nine amino acids are invariant and an additional 17 have conserved their hydrophobic or aromatic character. Gaps in the plastocyanin sequence are concentrated in two regions. Of the 25 deleted residues 12 are found in position 67–86 and 8 in position 27–39. No gap penalty was used which means that the number of gaps has been maximised. A reasonable gap penalty (2–5) will move residues 29, 31, 32, 69, 70, 74 and 82 and remove up to five gaps, but not influence the alignment around the more conserved regions 47–51 and 109–130.

The score for the alignment of the composite sequences was 7.3 s.d. above the mean of the scores for 20 randomisations. When the 72 pairwise comparisons of the azurins to the plastocyanins, together with two randomisations (compare Fig. 2), were repeated imposing the deletion pattern of Fig. 3, the means of the two distributions were now 200 units apart compared with the earlier 60 (Table 1). About 60 points in the earlier pairwise comparisons were a result of "better than real" alignment. The distribution of similarity along the ailgned composite sequences was obtained as normalised score differences, before and after 20 randomisations, for all possible spans of 11 residues. The mean deviation from random was 2.8 s.d. units, with a s.d. of 1.1 s.d. units. Highest deviation from random, about 4 s.d. units, was found around residues 15–25, 42–51 and 105–115.

The similarities found above, although very suggestive, do not by themselves prove the azurins and the plastocyanins to be homologous. A direct test for divergence was carried out using the most parsimonious phylogenetic tree, calculated according to Moore *et al.*[7], for the proteins in conjunction with the Fig. 3 alignment. The above-chance occurrence of identical nucleotides in the independently constructed sequences found by the parsimony procedure of Fitch[8] and Fitch and Farris[9] at the most ancestral node of each of the two groups of proteins, were then calculated[10]. The figure obtained, +6.9 s.d., strongly supports the possibility of divergent evolution.

Only fragmentary data are available for the other small blue proteins. Stellacyanin (C. Bergman and L Strid, unpublished) and umecyanin[11] have about 110 and 130 amino acids with a single cysteine residue plus one disulphide bridge. This bridge is not, however, positioned as the one found in the azurins as no cysteine is found in the first 30 positions in umecyanin, nor in the first 12 positions in stellacyanin. Also, they both have a considerable amount of bound carbohydrate. Their N-terminal sequences nevertheless show considerable similarities with each other and with the N-terminus of the plastocyanins (Fig. 4), indicating that possibly all the small blue proteins have a common origin.

Table 1 Means for alignment scores for the azurins compared with the plastocyanins

	Real sequences Mean score	Real sequences s.d.	Random sequences Mean score	Random sequences s.d.
All sequences (72 pairs)*	1,375	20.5	1,316	21.6
All sequences except prokaryotic plastocyanin (63 pairs)*	1,370	9 6	1,322	11.7
All sequences Given deletion pattern† (72 pairs)	1,318	27.9	1,117	31.0

* Optimal alignment.

† Alignment that is optimal for composite sequences (Fig. 3).

Scores were calculated before and after random permutation of one of the two sequences in a pair. Randomisation was done twice for each pair.

```
Plastocyanins  1    --LDVLLGGDDGSL
               2    --LEVL  SGD S
               3    --VEVL  ASD G
               4    --VEIL  GED S
               5    --IEVL  GDD S
               6    --VEVL  GGD S
               7    -DVTVK  ADS A
               8    ETYTVK  SDK L

Umecyanin           EDYDV--GGDDE
Stellacyanin        TVYTV--GDSAGWK
```

Fig. 4 N-terminal amino acid sequences of plastocyanins (Fig. 1) as compared with umecyanin[11] and stellacyanin (C. Bergman and L. Strid, unpublished).

The only known common function for the proteins considered is to bind copper in a very particular way. Residues involved in copper binding are presumably untouched by evolution and to be found among the unvaried (and hydrophilic) sidechains. These are (Fig. 3) His 47, Asn 48 and Cys 113. In addition His 118 in the azurins is unvaried but displaced one position in the plastocyanins. The unvaried Tyr 109 and Met 122 are possible but poor Cu ligands Spectroscopic data[12–14] indicate that both nitrogen and sulphur are ligand atoms and chemical evidence[15] points to Cys 113 as one of the ligands.

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matters arising

Trapa natans in the British Flandrian

I WOULD like to make a palaeobotanical comment and to cite some further literature in reference to the paper by Flenley *et al.*[1].

The distribution of isolated post-glacial subfossil finds of *Trapa* (their Fig. 2) can be supplemented by three pollen finds from southern Norway (east of Oslofjord[2]) and a macrofossil (fruit) find from just north of Gävle in eastern Sweden[3] (61 °N). At another, central Swedish site[4] pollen of *Trapa* was found to occur a little higher up the profile than fruits.

All these sites lie within the hypothetical climatic limit for *Trapa* during the climatic optimum (Atlantic and/or Sub-Boreal chronozones) drawn by Hintikka (ref. 5, Fig. 46), based on an extension of the present-day limits (mean temperature of the warmest month >17.5 °C and of the coldest month <1 °C) by Iversen's[6] postulated increase in mean July temperature of +2 °C. These values are corroborated by the meteorological data for subfossil and recent localities tabulated by Apinis[7], who in addition draws attention to the critical period in May–June, during which a water temperature on the lake bottom of >12 °C is necessary for seed germination, and of September–December temperatures of 10 °C–1 °C for successful fruit ripening. Hegi (Flenley's ref. 3) states that flowering does not occur at water temperatures < 20 °C.

Hintikka's fossil map only covers Fennoscandia, but comparison of the course of the 16 °C mean July isotherm[8] (interpolated for Fig. 9.2*a*) for recent time for Britain and western Denmark–southern Norway supports the possibility of subfossil occurrences of *Trapa* in Britain south of the Humber–Dee line (18 °C) in favourable aquatic habitats.

Habitat suitability needs stressing, however, particularly for outpost sites, as regards lake water depth, topography, sedimentation and water chemistry[7,9,10]. We are told too little about the site conditions at the Skipsea meres to draw any conclusions; or about any associated members of the aquatic flora (supporting macrofossil studies are required urgently!). The absence of subfossil finds of *Trapa* pollen or fruits at Hockham[11] or any other East Anglian meres could conceivably arise from their (generally lime-rich) eutrophy (compare the frequent finds of *Naias marina* seeds and Flenley's ref. 1, pp.100–102), or that they do not lie on swan/goose migration routes, although human influence during the Neolithic and Bronze Age periods would surely have secured the introduction of *Trapa* as easily there as on Humberside? The best correlation between prehistoric sites and former *Trapa* lakes has been presented by Sundelin's[12] investigations in Småland, central southern Sweden, in which the absence of *Trapa* fruits from lakes to the north of his area was ascribed to climatic causes. Comparison with Hintikka's map shows a postulated negative 'inlier' in just that position. *Trapa* and man were obviously coeval there, but without ^{14}C datings it is hard to be certain that man got there first.

Although not disagreeing with Flenley that man may have had a 'finger in the *Trapa* pie' (although the 'Swan-maiden legend' would explain both the Humberside and South Uist finds!), I cannot agree that "in which case it would not be necessary to invoke climatic change"; a more favourable local climate than that of the present day is a prerequisite for successful establishment and continued presence of *Trapa*. It is an annual and I can find no reference to overwintering by turions, only as fruits, although these remain viable for several years at least.

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FLENLEY AND MALONEY REPLY—We thank Tallantire for his valuable comments[1], and we are sorry that he read our paper to imply that we were trying to explain all the northern European Holocene records as resulting from human activity. As we said originally "explanations relating to man, however, deserve re-examination in the context of the new records".

In attempting to explain the new British records, we should like to make the following points. First, it is a general principle in ecology that a species cannot be assumed to occupy its full potential range in the field[2]. The entire ecological amplitude can only be established by comprehensive laboratory experiments. Although the experiments of Apinis[3] undoubtedly go some way towards this, they seem to relate more to specific phases (for example, germination, fruit ripening) than to the complete life cycle. Cultivated plants commonly occur well outside their 'wild' range.

Second, ecotypic differentiation may be expected to occur rather rapidly in an annual species, and the possibility of an extinct ecotype cannot be eliminated. Even in perennial species, ecotypic differentiation is very rapid[4].

Third, we have investigated, in addition to the two meres which did contain both *Trapa* pollen and Bronze Age dwelling platforms, four other north Humberside former meres from which Bronze Age dwelling platforms have not been reported, and we did not find *Trapa* pollen in any of these. Of course, it is difficult to argue from correlations based on absence, which is why we did not adduce this evidence in the original letter. We note, however, that Tallantire refers to the absence of finds of *Trapa* in East Anglian meres.

Fourth, there seems to be some similarity between the Bronze Age dwelling platforms of north Humberside and those of Scandinavia[5] suggesting a possible former cultural connection.

We would not like the foregoing arguments to be taken to imply that we necessarily prefer the hypothesis of human activity to that of climatic change in explaining the former occurrence of *Trapa* in north Humberside; we are simply suggesting that minds should be kept open. Still less would we like it to be thought that we are necessarily against the idea of a climatic optimum; we simply suggest that the new British *Trapa* records may not demand this as an explanation.

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R factor transfer *in vivo* in sheep with *E. coli* K12

IT has been inferred that the bacterium *Escherichia coli* K12 is a naturally "feeble" strain which can be used in some genetic engineering experiments with little concern that it might "escape" containing a reconstituted plasmid. Also, there seems some doubt as to whether *in vivo* plasmid transfer, especially from the commonly used *E. coli* K12 F^-, can occur to a significant extent within the gastrointestinal tracts of normal, adult humans or other animals[1,2]. This implies that even if a few organisms should be ingested by laboratory personnel, transfer of a plasmid to other bacteria would be negligible and the "feeble" *E. coli* K12 should be quickly eliminated from the human gastrointestinal tract.

In an attempt to ascertain how antibiotic-resistant organisms have become so widespread in farm livestock, I have studied the conditons under which R factors could be transferred from donor to recipient bacteria *in vivo* in sheep[3]. These experiments were repeated using donor and recipient strains of *E. coli* K12 with similar results. By imposing a short period without food (24–48 h duration) on these animals, the environment within the gastrointestinal tract was altered and the administered *E. coli* K12 strains were able to multiply *in vivo*, reaching a population density sufficient to donate or receive an R factor. An experiment was also conducted in which an R factor was transferred *in vivo* from a small number of administered *E. coli* K12 donor organisms (4.0×10^3 cells) to the natural coliform flora of an adult sheep where it survived for 7 d (in up to 10^5 wild-type cells per g faeces). In similar experiments transferring the same R factor from 'animal' donor strains to the natural microflora of sheep, coliform organisms containing the R factor were sometimes excreted for only a few days. At other times, however, these organisms could be excreted for weeks or even months afterwards. It is not known whether the particular donor organism has an effect on the duration of carriage in the microflora of an animal, but this seems unlikely.

These experiments were carried out in the absence of any form of antibiotic treatment, and possession of the R factor should have conferred no special survival value on the host bacterial cells. Using similar nutritional conditions, therefore, it may be possible to transfer other plasmids, perhaps even reconstituted ones, *in vivo* in sheep.

If *in vivo* transfer can be accomplished in one animal species using *E. coli* K12 organisms, it is likely to take place in other animal species, including humans. The difficulty will be to define the conditions in which it occurs.

Short starvation periods or other changes causing temporary alteration of the environment and microflora of the gastrointestinal tract may well potentiate plasmid transfer in monogastric as well as ruminant animals. It is in the interests of all concerned in genetic engineering that this possibility is recognised.

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Human ζ haemoglobin chain

THE embryonic ζ chain occurs in combination with γ chains in haemoglobin (Hb) Portland[1]. Lehmann[2] and Huehns[3] have suggested that the ζ chain is an α-type chain and attempted to confirm the idea by comparison of the amino acid compositions of the tryptic peptides from the ζ chain with those of the human α chain[4,5] and known mammalian α chains[6]. From similar data other workers have proposed the same α chain relationship for the corresponding x-chain found in other mammals[7]. The presence of a Tyr–Arg dipeptide[4,5], the absence of a D helix[4,6], the identification of residues which could participate in α–β contacts[5] and cooperative oxygen binding by Hb Portland[4–6] have been used to support the contention that the ζ chain is a primitive α chain.

I submit that the relationship between the ζ and α chains has not been clearly established by the published evidence. Tyr–His, present in non-α chains, could become Tyr–Arg as a result of a single base change. The missing D helix, like the detection of appropriate α–β contacts, could easily result from the deliberate attempt to align the ζ-tryptic peptide compositions with the α-chain sequence. Certainly the β_4 tetramer does not show cooperative oxygen binding but this does not preclude the possibility that some asymmetric tetramers with no α-chain content could have cooperative function.

Examination of the amino acid compositions of the various human Hb chains[1] by any of the proposed methods[8–10] suggests that the ζ chain is most closely related to the γ chain. The available tryptic peptide compositions of the ζ chain can be aligned with the γ-chain sequence to give 46 differences in 131 positions of comparison (the alignment includes a D helix). In the most recent comparison of ζ-chain peptide compositions and the α-chain sequence there were 47 differences in 135 positions.

I do not claim that the ζ chain is closely related to the γ chain. I do suggest that the lack of restraints in aligning tryptic peptide compositions within a family of related sequences can lead to erroneous and misleading conclusions, and urge that at least the tryptic peptide sequences be obtained before the principle of least dissimilarity is invoked and evolutionary or functional relationships proposed.

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LEHMANN REPLIES—The α and γ chains of man have more than 60% of their sequences in common. It is therefore not surprising that it is possible to align parts of the ζ chain with either. The fact remains that haemoglobin (Hb) Bart's (γ_4) shows no cooperativity, whereas Hbs F $(\alpha_2\gamma_2)$ and Portland $(\zeta_2\gamma_2)$ do. The cooperativity of Hb Portland is such[1] that it is difficult to visualise it without the conventional $\alpha_1\beta_2$ contacts typical of cooperative haemoglobins. The fact that it is possible to align by homology residues of the tryptic peptides of the ζ chain in a manner which places $\alpha_1\beta_2$ contacts in the expected positions fully justifies that exercise.

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Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

reviews

Diseconomics of deforestation

N. W. Pirie

Losing Ground · Environmental Stress and World Food Prospects. By Erik P. Eckholm. Pp 223. (Norton: New York, April 1976.) Cloth $7.95; paper $3.95.

DESERTS are expanding, hillsides washing away, rivers and reservoirs are silting up and the oceans are becoming polluted. Eckholm argues that the destruction of forests is the main cause of all but the last of these misfortunes. He states a convincing case and points out that it is not new. Hadrian tried to protect the cedars of Lebanon, and, at the end of the eighteenth century, deforestation was blamed for flooding around Grenoble and in Alpine valleys. As a result, reforestation programs were started in the Alps and Appenines.

Iron smelting was responsible for the later stage of deforestation in Britain: agriculture was responsible for the early stages. Eckholm says that more forest is now being destroyed to make farm land than to supply industrial timber, that the swidden (slash and burn) technique destroys more trees than settled agriculture, and that half the wood cut throughout the world is needed for fuel. Destruction for domestic fuel is not new. In four years after the French Revolution, 3.5 million hectares of forest that had been protected by aristocrats was destroyed. Now, 20–30% of the family income in some countries is spent on fuel, and wood costs as much as the equivalent amount of kerosine.

Oil prices are not likely to diminish; the demand for wood fuel will therefore increase in the less developed parts of the world and make planned forestry even more important, both to maintain supplies of wood and to prevent erosion. A survey is quoted showing an annual loss of a few kilograms of soil from forested hillsides, a few tons from pasture, but 8–100 tons from arable land. There is, however, no hope of improvement in methods of land use until the informed consent of the inhabitants has been won. A similar point arises when overgrazing creates deserts. If, as is stated, the Sahel would produce twice as much meat and milk if it carried half the present number of cattle, it should be possible to convince people of this. Before the commons were enclosed, our forefathers had controlled the number of animals each person had the right to graze. If we could do it, others can.

Eckholm does not want to preserve tropical rain forest for sentimental reasons, nor does he support the mistaken idea that forests are a better source of atmospheric oxygen than other forms of plant cover. But he quotes examples of the failure of attempts to replace it by 'western'-type farming, and argues that that approach is never likely to succeed. He is no more hopeful about the more sophisticated forms of swidden that are grouped under the term 'corridor' systems.

Clearly, the ground should seldom, if ever, be left bare. This means that perennial crops will be the mainstay of satisfactory farming in rain forests Rubber and oil palms are traditional. The products from these crops, as of many other tropical crops, move into world trade; a variable, but usually small, fraction of the money earned moves back to the primary producers. They would fare better if they could grow crops that yield a balanced diet for local use. There will be a revolution in agriculture in the wet tropics when perennial plants are developed from which food with a reasonable protein content can be prepared by simple techniques.

This is a popular account of our mismanagement of Earth's biological resources. British readers should remember that Americans think a 'watershed' is a valley; scientific readers may be irritated by the repetitions, the 'purple passages', and the author's ignorance of the meanings of such words as decimate, hopefully, ineluctable and sedimentation. Scientists will, however, welcome the impressive bibliography—30 close-packed pages of references and suggestions for further reading. ☐

Norman Pirie has recently retired as head of the department of biochemistry at Rothampsted Experimental Station, Harpenden, UK.

Forest energy for half the world

Forest Energy and Economic Development. By D. E. Earl. Pp. vii+128. (Clarendon Oxford; Oxford University: London, October 1975.) £5

WHEN we run out of oil and coal can we use wood? This small book, part of an Oxford series on Science Policy, will be a good start for debates on long-term substitutes for the alarmingly expensive and diminishing fossil fuels.

Dr Derek Earl's answer to the energy crisis is that replacement of fossil fuels by wood is feasible now in countries which have a low per capita demand for energy, a small population and plenty of forest land. Good planning and management are essential, for without control, wood is as temporary a resource as oil or coal. Many developing countries are heading the same way as Europe, which has long since destroyed much of its forests In most of the richer, highly developed countries forest energy seems to offer little relief from the impending shortage of fuel unless the population is reduced and people can be persuaded to want less energy, a task more appropriate to poets and philosophers than to forest economists.

The statement in the preface that "the world's forests incorporate solar energy into organic material . . . at an annual rate far in excess of the world's present economic needs" is shown later to be almost irrelevant compared with the practical tasks of growing, harvesting, processing, transporting and marketing forest fuels cheaply

After preliminary chapters putting economic growth and energy resources into perspective, most of the book is devoted to ways of supplying firewood and charcoal for particular situations in developing countries, including several case studies from the author's own wide experience He has a particular interest in the technology of charcoal production which is well described

Half the wood harvested in the world is used for fuel and about half the people of the world depend more on wood and charcoal for their cooking and heating than on other sources of energy. Of the world's total recorded annual consumption of energy from all sources, 7% is from wood. In such countries as the US and the UK, less than 1% comes from wood, but in Tanzania, Nigeria, Nepal and several other countries the proportion is over 90%.

The book is not difficult reading for the non-specialist as the author explains many of the basic concepts of both forestry and economics. His efforts to present ideas and data in a simple direct style, however, have resulted in a somewhat uncritical treatment.

As a source of data for discussion or decisions the book is only as good as its sources which the author acknowledges to include unreliable estimates. Who can record accurately the 'theft' of firewood gathered by people who regard forests as God-given and bountiful? A determined survey of consumers in one country showed that only 1.5% of fuelwood used had been recorded previously in government trade statistics.

The renewability of forests is a key point in the book. At the common yield of about 3–6 tonnes per hectare per year fuel wood production has not often been a profitable use for land. The occasional yields of eucalypt plantations of more than 40 tonnes per hectare per year on the best sites, however, are highly profitable. Can the yield be increased economically on other sites?

The current low prices of fuel wood are partly based on the wrong assumption that a forest costs nothing to grow or replace. With this assumption wood is mined, dissipating the energy capital of forests. Growing fuel wood as a crop will become a legitimate use for valuable land as the price and undesirable social costs of oil and its substitutes rise with scarcity. This is Earl's economic theme, the effects of depletion and scarcity on the price of fuel.

Earl's book may be as he says the first to assess the potential of the world's forests "as major stores and suppliers of energy". It is unlikely to be the last on this increasingly urgent subject.

Ken Eldridge

Soviet geological cruises

Rift Zones of the World Ocean. Edited by A. P. Vinogradov and G. B. Udintsev. Pp. viii+503. (Halsted Wiley: New York and Chichester; Israel Program for Scientific Translations: Jerusalem, December 1975.) $49.50; £24.75.

DON'T rush straight off to the bookshop, all of you eager to join in the IPOD research program. Although its title suggests a quick way of catching up on what went on in the ocean crust while you were doing your lunar rocks, this book is not that at all. First published in Moscow in 1972, with Soviet references up to 1970, and foreign references to about 1968, this is a compilation of Soviet articles about two geological research cruises in the Indian Ocean in 1964 and 1967.

I stress the dates because it is most striking as a historical document. At that time, there was an equivocal relationship between Soviet marine geology and plate tectonics. A year or so before, diverging arrows had begun to appear on Soviet diagrams of mid-ocean ridges, although the text was usually reticent about their precise significance. Here, the silence is profound in most of the book, most notably in the chapter on magnetics, but in the last chapter a clear acknowledgement of oceanfloor spreading as a possible process occurs both in a diagram and in the text.

Since most of the new ideas from this research have already found their way into the scientific consciousness, becoming transformed in the process, why should one buy the book? Not for its diagrams or the precision of its writing, both about par for Soviet texts. But the chapter on the 1967 seismology still looks modern, and there is one too on mineralisation in which two small sulphide-bearing rocks are described. The large collection of ultramafic rocks is well described, and there are a fair number of major element analyses, but the usefulness of the book as a quarry for data is severely reduced by the lack of any detailed positions within each polygon for the dredge stations.

I'm sure that if Gleb Udintsev and his colleagues were writing it again now, they would write it very differently, so that it is hard to recommend it enthusiastically now as a newly published book.

J. R. Cann

Bio-inorganic chemistry

An Introduction to Bio-Inorganic Chemistry. Edited by David R. Williams. Pp. 402. (Thomas: Springfield, Illinois, January 1976.) $24.50.

THE preface states that this book was "conceived in a bar on Amsterdam airport by an *ad hoc* committee of inorganic researchers delayed by a pilots' strike". Conception in such circumstances might well be expected to produce a somewhat unselected set of characteristics in the offspring. Now that the child has been delivered we can view the baby and, mercifully, although it has the predictable blemishes, it has many endearing features. The worst blemish it shares with its parents is the wish to be too many different things, clearly shown by the section headings: bio-inorganic chemistry; instrumental approaches to bio-inorganic chemistry; biomedical applications. Taking these in turn, we might have expected first, an orderly introduction to the subject but in its place we have a jumbled set of chapters, each good in its own right, on metal complex formation, oxygen and nitrogen, iron, calcium and magnesium, and on metalloenzymes but nothing on nucleotides sugars and membranes.

Section II is similarly disjointed. It is good on thermodynamic studies but there is no coverage of spectroscopic, magnetic, electron microscope probe methods, and so on. Bio-medical applications is a different brew but it has very little information on, for example, the effect on disease of element concentrations, although it does introduce many risky topics—for example, cancer, death, and so on (everlasting life escapes). This is a very complicated area of biological science and I am not sure that a superficial taste of some of it is not harmful or even addictive for readers never mind some of the authors.

In previous reviews of books on bio-inorganic chemistry, I have pointed to the absence of material on Na/K/Mg/Ca metabolism. This fault is remedied in this book. I now point to another defect of conventional bio-inorganic chemistry which is strikingly apparent here—the absence of reference to the role of non-metals, B, Si, P, S, Cl, Se, Br, I, and so on. This could easily be put right in a future edition.

The overall impression given by this book is of a subject, a very large subject, in the act of growing up. Bio-inorganic chemistry as seen here is beginning to receive an education, although it is still necessary for parts of it to learn their proper functions. Surely a major purpose of the book is achieved in directing attention to what inorganic chemistry is. Inorganic chemists must cease to occupy themselves with toy-rattles making noises about molecular spectroscopy and magnetism. They must again turn to the study of *chemical* properties. As this book shows, with its accent on enthusiasm, one big exciting area of inorganic chemistry is to be found within biology and much of importance remains to be discovered.

R. J. P. Williams

Carbonate petrology

Carbonate Facies in Geologic History. By James L. Wilson. Pp. xiv+471 (30 plates). Springer: Berlin and New York, 1975). DM 90; $36.90.

Carbonate Facies in Geologic History is not written with the precision of Henry James, but neither is it as hard to read as *Finnegan's Wake.* Although several readings are required for understanding of many parts, this book is the kind of helpful work that would require close study even if the prose were pure.

Wilson's approach is inductive. He cites with approval Mark Twain's conviction, after viewing thousands of European cathedrals, that he should study another 97,000 or so before making up his mind on their aesthetic value. Geology, however, has always been the art of drawing sufficient conclusions from insufficient premises—the Samuel Butler view of life.

One of the biggest surprises is Wilson's decision (p10) to use Dunham's rather than Folk's classification ". . . to avoid the necessary rigidity involved in applying Folk's particle-type prefixes and because the packing concept of Dunham is important and should be designated by a primary name." I'm uncertain of Wilson's meaning, but for most carbonate petrologists the descriptive nature of Folk's classification and its applicability to environmental interpretation have made its use appropriate for many diverse problems.

The first chapter is a short outline of the principles of carbonate deposition. Chapter two reviews stratigraphic and facies patterns and paleotectonic settings. An outline of carbonate petrography is given in chapter three where useful descriptive techniques for environmental interpretations are stressed. The remaining chapters (4–11), except for a concluding summary (chapter 12), contain descriptions of many examples of marine carbonate facies.

In the descriptive chapters Wilson briefly outlines the tectonic setting, the geologic history, and the stratigraphy. Depositional and diagenetic factors that have led to the development of good porosity and permeability are stressed. Many characteristics of carbonate facies that are helpful in recognising favourable areas for petroleum exploration are included.

This book is of greatest value to carbonate petrologists engaged in petroleum exploration. The treatment is too detailed for undergraduate students, for whom it was not intended, and will even present difficulties for many graduate students and petroleum geologists for whom the book was written. A considerable amount of experience in diverse marine carbonate sequences is required for comprehension, especially a good background in paleontology and carbonate petrography.

At $36.90 no student wil rise early, eat a roll, brush his teeth, and stroll to the bookstore for a copy. Not willingly, at any rate. Few teachers not independently wealthy will wend their way to the cashier with a copy. If the book sells well enough for the publisher to issue a paperback edition at half this price, many individuals will buy it because this is a useful volume. Wilson has had an outstanding career in carbonate petrology; much of his knowledge is presented here.

M. Dane Picard

Fluorescence spectroscopy

Biochemical Fluorescence: Concepts. Vol. 1. Edited by Raymond F. Chen and Harold Edelhoch. Pp. xii+408. (Dekker: New York, August 1975.) $29.50.

Fluorescence spectroscopy is to many biochemists a Jekyll and Hyde affair. The classical uses of the technique—for example, histological staining, estimation of metabolite concentrations and of enzymic activities—are simple in application, and the results obtained easy to interpret. This situation began to change in the 1950s with the increasing sophistication of solvent shift and polarisation studies, although most biochemists could still appreciate the conclusions of such studies and their relevance to biochemistry even if they did not understand the physical basis of the techniques. Over the past decade, however, the subject has undergone a further transformation such that totally new aspects—for example, circular fluorescence polarisation—are, at least within the confines of a scientific paper, beyond the grasp of most biochemists not working in the field.

This book will do a great deal to alleviate the problem. It consists of seven chapters, each devoted to a basic concept or application of fluorescence spectroscopy and written by one or more leading authorities in that area. The general topics covered are fluorescence decay, fluorescence polarisation, energy transfer and kinetic techniques utilising a change in the fluorimetric properties of the reactants. This volume is devoted solely to theory and instrumentation. The applications to biological systems will be covered in volume 2. Nonetheless, a biochemist interested in understanding the basis of fluorescence techniques could read this book with advantage. Each topic is developed from a simple description of the physical processes involved to the level needed to understand the latest publications. The extent of the mathematical development included is just about right to maintain continuity without overburdening the reader who does not want a rigorous proof of each step. Such a treatment, necessarily, leaves many logical gaps which, if the book is well referenced, the interested reader can fill in himself. In places, the references of this otherwise useful book falls short of that which will enable him to do this easily. The extensive treatment of several areas beyond that found in the literature will make this an attractive book for workers in the fluorescence spectroscopy field. This is especially true of the Dale and Eisenger article on energy transfer which takes up two-fifths of the book and half of which is devoted to a graphical treatment of the vexed orientation factor.

The contributors—especially Steinberg—should be commended for their honesty in clearly stating the problems of applying the techniques to biological systems and in outlining the areas in which present theory does not adequately explain the experimental results.

M. T. Flanagan

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London's Zoo

London's Zoo. (An Anthology to Celebrate 150 Years of the Zoological Society of London, with its Zoos at Regent's Park in London and Whipsnade in Bedfordshire.) Compiled by Gwynne Vevers. Pp. 159. (Bodley Head: London, Sydney and Toronto, April 1976.) £4.95.

Bear pit (1835) by George Scharf

Zoological Society of London (Michael Lyster)

WHAT a great deal of fun Dr Gwynne Vevers must have experienced sifting through the enormous amount of material available to him and what frustrations he must have experienced when it came to editing, perhaps one should say culling, the plethora of quotable quotes that he must have gathered together. While reading the book one soon becomes aware of the careful thought which has gone into the form of presentation. Inevitably he has concentrated on the early days, days when the foundations of the Zoological Society of London were being laid with a clear emphasis on the advancement of zoological science and the development of a living collection of animals. In those days creatures arriving at the Society's premises might be found in a crate, a jar of spirit, or just a package of skin and skeleton for examination by the early zoological researchers. The Society's museum was consolidated in 1849 with the Zoology Department of the British Museum, and since that date material available as a result of deaths in the animal collection has gone to this museum.

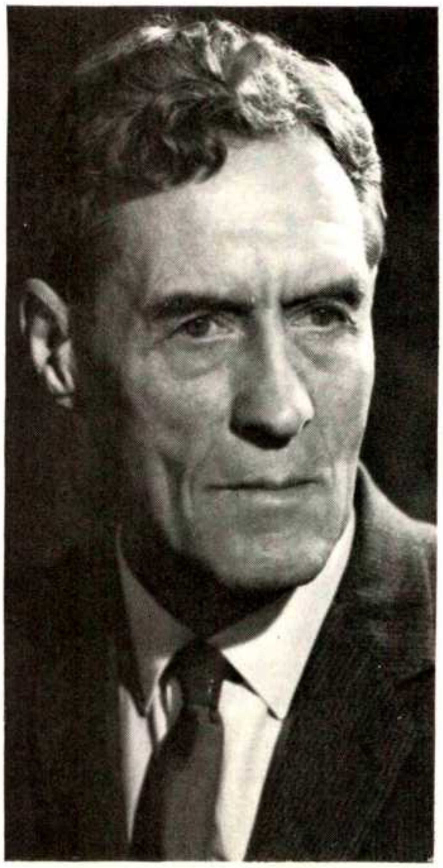
It was this insistence by the founding fathers on a scientific approach to the development of its collection that has resulted in the Society being held in the highest esteem in the zoological world. But to the millions of visitors who have visited the animal collection over the years it has been the "Zoo", a place to wonder at strange creatures, to be amused, to recreate, to escape for a moment from the sometimes harsh realities of everyday existence.

The anthology is the Zoo itself, it involves animals and people, ideas and ideals, the serious, the flippant, the scientific and the anthropomorphic. It should make good and enjoyable reading for all who have a specific or passing interest in the growth and development of this great Zoological Society and its Zoo.

R. J. Wheater

Lovell on Blackett

P. M. S. Blackett: A Biographical Memoir. By Sir Bernard Lovell. Pp. 115. (Royal Society: London, 1976.) UK £4; Overseas £4.10.


Lord Blackett

THIS book is a reprint of Bernard Lovell's account of Lord Blackett published recently in the *Biographical Notices of the Royal Society*. These *Notices* are accounts of recently deceased scientists written by their friends. They are of very great interest now and will be invaluable to historians in the future. Regrettably few libraries, especially in the US, subscribe to the series. The Royal Society wisely insists that the *Notices* be written within a few months of the subject's death and usually gets an account that bears the impress of friendship and of recent memories of personality and oddities of character.

It is not easy to write a biographical notice: on the one side there is the pressure of friends, relations, and often of the widow, to omit anything at all colourful (a widow once insisted that I omit the fact that a man had died of a stomach haemorrhage after a lunch to celebrate his 90th birthday). On the other side there is the difficulty of giving a realistic account of scientific innovation and its attendant struggles, a thing done with such *éclat* by James Watson, and above all of letting the character emerge from action and anecdote rather than from explicit description.

In writing of Patrick Blackett all these difficulties are magnified by the immense achievement. The writer could easily be overwhelmed by the ramifications of the three careers, in science, in warfare and in politics and by the major achievements in three fields of science—nuclear physics, cosmic rays and geomagnetism. In fact Lovell has done it superbly well. The account is beautifully clear and readable, and Blackett emerges from it as the uniquely intelligent and humane figure that he was. It is a book that every young physicist should own and that no-one who knew Patrick could possibly be without. The immense care that has gone into getting it all right has been enormously worthwhile. This is how Blackett appeared to his contemporaries and anyone who writes about him in the future must rely largely on this.

Edward Bullard

obituary

Gordon Miller Bourne Dobson, CBE, ScD, FRS, former reader in meteorology in the University of Oxford, died at the age of 87 on March 10. After school at Sedbergh he went up to Gonville & Caius College, Cambridge. The post he held from 1911–1913, as student-assistant at Kew Observatory, followed by the Lectureship in Meteorology at the Central Flying School at Upavon, established the broad interest in the physics of the atmosphere which dominated his long and productive life. During his period at the Royal Aircraft Establishment, Farnborough, in the First World War, he was invited by Professor F. A. Lindemann to join him at the Clarendon Laboratory, Oxford, where he was appointed University Lecturer in 1920.

Their discovery, from the study of meteor trails, of a warm region in the atmosphere at heights of $\sim$50km, and the inference that the source of heat must be the absorption of solar ultraviolet radiation by ozone, set Dobson on the researches which he carried out so energetically and fruitfully to the end of his life: the physics of the stratosphere in general and in particular the behaviour of ozone.

Fortunately Dobson has left us with a full and entertaining account of his own work up to 1966: Forty years' research on atmospheric ozone at Oxford: a history, *Applied Optics*, 7, 387–405 (1968). After that date he continued making substantial contributions to the study of atmospheric ozone, involving thousands of observations on 'No. 1', the prototype photoelectric photometer built with his own hands in 1927 and only slightly modified since (photomultiplier and electronics). His work was done almost entirely at his home, and to a great extent financed out of his own pocket. There was a regular pattern of morning work, in the study if necessary but preferably in the workshop or observing hut, gardening in the afternoon (all weathers), further work after tea and reading (mainly scientific but in his last years general and voracious) after supper. If this regime sounds monastic, he was no hermit, and visitors to Watch Hill were always welcome; after tea or morning coffee Dobson was always eager to discuss some new observations, demonstrate some new apparatus, or discuss the recent literature.

His gift of going directly to the heart of a problem made him an outstanding speaker and his enthusiasm was infectious: his lectures on the atmosphere at the Clarendon Laboratory were always packed.

In the ozone community he seemed a permanent fixture and we are still staggered by his leaving us; for those who were privileged to work closely with him there are treasured memories and deep gratitude for his life and example.

C. D. Walshaw

Karl Weissenberg was born in Vienna, Austria on 11th June 1893 and attended many different schools in Austria, Germany and France before matriculating in Frankfurt a/Main at the age of sixteen and a half. He subsequently studied at the Universities of Vienna, Berlin and Jena, taking Mathematics as his main subject, with Physics and Chemistry as subsidiaries, touching also upon Law and Medicine. He became a Privat-Dozent and, later, Professor of Physics at the University of Berlin.

In 1922 he joined the research team of M. Polanyi at the Kaiser Wilhelm Institute für Faserstoffchemie in Dahlen, Germany, and there, for a period of about six years, worked on X-ray crystallography. His fame in this field rests on his invention of a new X-ray goniometer, which allowed for the first time, a unique determination of 3-dimensional crystallographic structure. The idea was only slowly adopted, and it was not until after the war that the Weissenberg X-ray Goniometer, as it is known, came into general use. By 1929 his attention turned from crystallography to rheology and he presented a general analytical method for finding the shear rate at a capillary wall, now widely used.

Just before the war, Weisenberg took up residence in Britain, and his wartime work on flame thrower fuels stimulated his curiosity and interest in the so-called anomalous liquids, and hence in the field of liquid rheology itself. He also demonstrated, by means of rubber and fabric sheets, the importance of rotation of the embedded co-ordinate system in a flowing continuum. He had much earlier conjectured that certain fluids were elastic and now came the inference of the existence of a tension along the streamlines, since amply demonstrated. At this time, too, the essence of the Weissenberg Rheogoniometer was established. The principle behind this second major invention was that it allowed the stresses around the full solid angle in a flowing continuum to be experimentally determined.

In addition to a deep and sensitive appreciation of a range of natural phenomena, Karl Weissenberg was an entirely engaging and unselfish person. His accord with nature was faithfully mirrored in that with his fellow human beings. He was notable in his scientific achievements and noble in personal qualities.

John Harris

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announcements

Awards

The American Society for Microbiology has awarded:

The **Wyeth Award** in Clinical Microbiology to **Edwin H. Lennette** of the California Department of Health.

The **Eli Lilly and Company Award** to **Ronald W. Davies** of Stanford University School of Medicine.

The **Carski Foundation Distinguished Teaching Award** to **Elizabeth R. Hall** of Washington State University.

The Biochemical Society has given:

The **BDH Award in Analytical Biochemistry** to Professor John Landon of St Bartholomew's Hospital.

The **Colworth Medal** to Dr W. J. Brammar of Edinburgh University.

The **Keilin Medal** to Dr S. M. E. Magnusson of the University of Aarhus.

Meetings

July 7–9, **Annual Meeting of the Biochemical Society,** London (The Meetings Officer, The Biochemical Society, 7 Warwick Court, High Holborn, London WC1R 5DP).

June 23, **Nuclear Medicine Imaging Systems,** London (British Institute of Radiology, 32 Welbeck Street, London W1M 7PG).

October 12–15, **Evolution and Lateralisation of the Brain,** New York (Conference Department, the New York Academy of Sciences, 2 East 63rd Street, New York 10021).

August 30–September 3, **The Chemistry and Uses of Molybdenum,** Oxford (Molybdenum Conference, Climax Molybdenum Co. Ltd., Villiers House, 41/47 Strand, London WC2N 5JS, UK).

September 21–23, **Autumn Meeting,** Sheffield (Dr John F Gibson, The Chemical Society, Burlington House, London W1V 0BN).

Reports and publications

Great Britain

Forestry Commission Forest Mensuration Handbook. (Forestry Commission Booklet No 39) By G J Hamilton. Pp. 274. (London Her Majesty's Stationery Office, 1975.) £4 00 net. [162

HMSO Subject Catalogue No 1. Statistics. (Publications of UK Government Departments and International Organisations. Pp. iii + 55. (London HMSO 1975.) [172

Universities Research Reactor. Annual Report, 1974–75. Pp. iii + 36 (Warrington Universities Research Reactor, Risley, Warrington, 1976.) [172

University of Glasgow The Hannah Research Institute: for Studies Relating to the Production and Utilization of Milk, Report 1975 Pp. 71. (Ayr: The Hannah Research Institute, 1976.) £1 00. [192

The Year Book of the Royal Society of London 1976 Pp. 425. (No. 80). (London The Royal Society, 1976.) £3 20. [232

Department of the Environment. Sixteenth Progress Report of the Standing Technical Committee on Synthetic Detergents Pp. v + 20 (London HMSO, 1976) 65p net. [242

Royal Observatory, Edinburgh. Annual Report, 1974/1975. Pp. 29. (Edinburgh Royal Observatory, 1976.) [242

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol 273, No 926 The Postcranial Skeletons of the Triassic Mammals *Eozostrodon*, *Megazostrodon* and *Erythrotherium* By F. A. K. Jenkins and F. R. Parrington Pp. 387–431 + Plates 1–6. UK £3.45; Overseas £3.55. Vol 273, No. 927 A Discussion on Water Relations of Plants. By J. E. Monteith and P. W. Weatherley Pp 433–618. UK £9.75, Overseas £10. (London The Royal Society, 1976.) [262

Agricultural Development Advisory Service—Ministry of Agriculture, Fisheries and Food, Wildlife Conservation in Semi-Natural Habitats on Farms A Survey of Farmer Attitudes and Intentions in England and Wales. Pp viii + 69. (London. HMSO, (1976) £1 25 net. [262

Scottish Development Department Towards Cleaner Water 1975—Report of a Second Rivers Pollution Survey of Scotland Pp. 72 + maps A–G. (Edinburgh and London. HMSO, 1976.) £5.90 net. [262

British Antarctic Survey Scientific Reports. No. 54 The James Ross Island Volcanic Group of North-East Graham Land By Dr P. H H. Nelson. Pp 62 + 11 plates. £6 net No. 90 Measurements of Atmospheric Ozone at the Argentine Islands and Halley Nay, 1957–72. By J C. Farman and R. A Hamilton Pp 40. £2 90 net (Cambridge: British Antarctic Survey, 1975) [272

Other countries

Energy: The Case for Conservation. By Denis Hayes. (Worldwatch Paper No 4) Pp 77 (Washington, DC: Worldwatch Institute, 1776 Massachusetts Avenue, N.W., 1976.) $2. [22

Republic of the Sudan. Ministry of Agriculture—Agricultural Research Division Annual Report of Yambio Research Station, 1963/1964 Pp. 19. (Wad Medani Gezira Research Station, 1975.) [32

CERN—European Organization for Nuclear Research CERN 75–20· The Design of the Control System for the SPS. By M. C. Crowley-Milling. Pp. 35 (Geneva: CERN, 1975.) [42

Royal College of Forestry, Stockholm. Studia Forestalia Suecica. No. 127 A Comparative Study of Chloroplast Morphogenesis in Seedlings of Some Conifers (*Parix decidua*, *Pinus sylvestris* and *Picea abies*). By Bjorn Walles and Jan Hudak Pp 21 No. 128: Influence of Photo- and Thermoperiod on the Initial Stages of Frost Hardening and Dehardening of Phytotron-Grown Seedlings of Scots Pine (*Pinus silvestris* L.) and Norway Spruce (*Picea abies* (L) Karst.) By Aron Aronsson. Pp. 20 No 129· Estimating the Accuracy of Site Index Curves by Means of Simulation By Bjorn Hagglund. Pp 34. (Stockholm Royal College of Forestry, 1975.) [62

Energy, Mines and Resources Canada Geological Survey of Canada Paper 76–1A· Report of Activities, Part A. Pp. ix + 522 (Ottawa: Information Canada, 1976) $6. [62

Energy from Thermonuclear Fusion. Pp. 48. (Garching bei Munchen Max-Planck-Institut fur Plasmaphysik, 1976) [92

Some Aziridines, *N*–, *S*– & *O*–Mustards and Selenium. (IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Vol 9.) Pp. 268 (Lyon International Agency for Research on Cancer, 1975 Distributed by World Health Organization, Geneva and HMSO, London). Sw.fr.27. [92

United States Department of the Interior. Geological Survey. Bulletin 1388 Influence of Rainfall and Ancient Landslide Deposits on Recent Landslides (1950–71) In Urban Areas of Contra Costa County, California. By T. H. Nilsen and B Turner, Pp. iii + 18 + plates 1 and 2. Professional Paper 774–C Mount Abbot Quadrangle, Central Sierra Nevada, California—Analytic Data. By John P. Lockwood Pp. iii + 18. (Washington, DC US Government Printing Office, 1975) [102

Biological Peroxidation of Lipids and Membranes. By M. Wolman. (Supplement to Vol. 11, *Israel Journal of Medical Sciences*.) Pp. 248. (Jerusalem. *Israel Journal of Medical Sciences*, 1975 [122

Geophysical Institute, University of Alaska Annual Report 1974/1975. Pp 183. (Fairbanks, Alaska Geophysical Institute, The University, 1975.) [122

United States Department of the Interior Geological Survey. Professional Paper 726–E Gravity and Aeromagnetic Study of Part of the Yakima River Basin, Washington. By S L. Robbins, R J. Burt and D O. Gregg. Pp iii + 7 + plate 1 Professional Paper 743–D. Dimorphism in Two New Genera of Devonian Tabulate Corals. By William A. Oliver, Jr Pp. iii + 11 + plates 1–7. (Washington, DC US Government Printing Office, 1975.) [122

Canada. Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 246 Sedimentology of the Archean Yellowknife Supergroup at Yellowknife, District of Mackenzie. By John B. Henderson. Pp. ix + 62 $6 Bulletin 247 Triassic Rocks of the Rocky Mountain Foothills and Front Ranges of Northeastern British Columbia and West-Central Alberta. By D. W. Gibson. Pp. 61 (9 plates). $5 Bulletin 251: Investigations of Lower Paleozoic Geology, Foxe Basin, Northeastern Melville Peninsula, and Parts of Northwestern and Central Baffin Island. By H. P Trettin Pp. 177. $10. (Ottawa Information Canada, 1975.) [122

United States Department of the Interior. Geological Survey. Professional Paper 729–D Geology of Sedimentary Rocks in Southern Yellowstone National Park, Wyoming. (Geology of Yellowstone National Park) By J D Love and W. R. Keefer Pp x + 60. (Washington, DC. US Government Printing Office, 1975) [162

University of California. Lawrence Berkeley Laboratory. Research Highlights, Fiscal 75. Pp. 75 (Berkeley, California. Lawrence Berkeley Laboratory, 1975.) [172

US Department of Health, Education and Welfare. Health Manpower in the Changing Australian Health Services Scene. By Ruth Roemer and Milton J. Roemer Pp. viii + 87. (Springfield, Virginia. National Technical Information Service.) [172

Environment Canada. Fisheries and Marine Service Technical Report No 584. Intestinal Trematode Parasites (Trematoda· Digenea) and Food of Yellowtail Flounder (*Limanda ferruginea* (Storer 1839)) from the Scotian Shelf and Gulf of St. Lawrence. By J. S. Scott Pp. iii + 12. Technical Report No. 593. Diogenetic Trematode Parasites and Food of Witch Flounder (*Glyptocephalus cynoglossus* L.) from the Scotian Shelf and Gulf of St Lawrence By J S Scott. Pp iv + 9. Technical Report No 594 Catch Statistics for the Bay of Fundy Herring Fisheries 1963–1974. By D. S. Miller and T D. Iles. Pp. iii + 75 (St Andrews, N.B.· Department of the Environment Fisheries and Marine Service, 1975.) [182

Smithsonian Contributions to Zoology, No. 203. New Species of Marine Molluscs from Pitcairn Island and the Marquesas. By Harald A. Rehder and Barry R. Wilson Pp iv + 16 (Washington, D C. Smithsonian Institution Press, 1975. For Sale By US Government Printing Office.) [182

Department of Energy Mines and Resources. Geological Survey of Canada Paper 74–47 Upper Paleozoic Rocks of the Atlin Terrane, Northwestern British Columbia and South Central Yukon By J. W H. Monger Pp. vi + 63 + 5 maps. (Ottawa Information Canada 1975.) $3 50, other countries $4.20 [202

United States Department of The Interior Geological Survey. Bulletin 1383–B· Upper Pleistocene Pyroclastic-Flow Deposits and Lahars South of Mount St. Helens Volcano, Washington. (Geology of Mount St. Helens Volcano, Washington) By Jack H. Hyde Pp iv + 20. (Washington, DC US Government Printing Office, 1975.) [202

Anuario del Observatorio Astronomico de Madrid para 1976 Pp.422 (Madrid·Observatorio Astronomico, 1975.) [232

Criterios Sobre Politica Cientifica/Criteria on Science Policy. (International Meeting on the Organization of Science, Madrid, 11–14 de Abril 1973. Actas Completas.) Edicion preparada por Roman de Vicente Jordana. Pp. 271 (Madrid Consejo Superior de Investigaciones Cientificas, 1975.) [232

National Research Council Canada Associate Committee on Scientific Criteria for Environmental Quality—Status Report Pp 68. NRCC No 14109. Waste Heat in the Aquatic Environment. By D. R. Dickson Pp. 39. $1. (Ottawa Publications, National Research Council Canada, 1975) [232

Mitteilungen aus der Biologischen Bundesanstalt fur- Land und Forstwirtschaft, Berlin-Dahlem. Heft, 166. Resistenzbildung gegen systemische Fungizide (Benzimidazolderivate) bei Colletotrichum Lindemuthianum (Sacc. et Magn) Bri. et Cav. Von Dr Ehler Meyer Pp. 135. (Berlin-Dahlem: Biologisches Bundesanstalt fur Land- und Forstwirtschaft, 1976.) DM 16.60. [242

Person to Person

Wanted: A copy (preferably hardback) of 'The Discovery of Time' by Toulmin and Goodfield (Hutchinson, 1965). Please contact Dr Peter J. Smith, Department of Earth Sciences, The Open University, Walton, Milton Keynes MK7 6AA, England, giving price, condition, etc.

The New York Academy of Sciences seeks, by July 1, nominations for its annual awards from individuals or organisations. For futher information write to. The Chairman of the awards committee, The New York Academy of Sciences, 2 East 63rd Street, New York, New York 10021.

A symposium entitled, 'Pesticide Induced Delayed Neurotoxicity', jointly sponsored by the US Environmental Protection Agency and the US National Institute of Environmental Health Sciences was held in Washington, DC, February 19–20, 1976. The full text of the papers and ensuing discussions of the basic scientific and regulatory aspects of organophosphate-induced delayed neurotoxicity are to be made available free to interested individuals and institutions. All inquiries should be addressed to Dr R. L. Baron, Health Effects Research Laboratory, Environmental Protection Agency, Research Triangle Park, North Carolina 27711.

The André Lightwitz prize (8,800 francs) is awarded to French or foreign scientists who have specially distinguished themselves in research on calcium and phosphorus metabolism, whether in the clinical or more fundamental fields. Conditions of entry for the 1976 prize can be obtained from INSERM. Applications must reach the Director of the INSERM (c/o Dr Lucienne Bonnot, 101 rue de Tolbiac, 75645 Paris cedex 13, France) by June 30, 1976.

There will be no charge for this service Send items (not more than 60 words) to Martin Goldman at the London office The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

nature

June 3, 1976

UN prepares for the next round

It is a safe bet at this time of Habitat that few people, even those concerned with United Nations affairs, have ever heard of the CSTD. The acronym stands for the Committee on Science and Technology for Development. At present, unlike many such international groups, the CSTD provides a forum for debate, rather than the sort of political battlefield which is now all too familiar. But the protagonists are the same: on the one side the countries of the Third World, on the other the industrialised, affluent countries. At the CTSD's third meeting, in New York earlier this year, the debate was concentrated for something like half the 3-week session on a single issue: who should run the United Nations Conference on the Application of Science and Technology to Development, now scheduled to take place, probably in Vienna, in 1979.

Even the proposal for this event has received hardly any publicity, inside as well as outside the UN; but enough information is now available to indicate certain differences from previous gatherings in the long series that started, in effect, with the first Atoms for Peace Conference in 1955. Probably the most important point is that from the start this has been intended as an inter-agency conference, the UN itself playing a central but much smaller role than hitherto. This should have the immediate advantage that the specialised agencies, almost all of which have a stake somewhere in the field of applied science or technology, need not feel that the meeting will be dominated by the political preoccupations of the UN.

At the same time, it is impossible to envisage the preparation for such a conference without continual and probably bitter in-fighting between the specialised agencies themselves—as witness the unheralded personal appearance of Mr M'Bow, the Director General of UNESCO, to ensure that his agency obtained a fair hearing at the recent session in New York. Probably one reason for this was that the organisation principally interested in the conference appears to be not one of those concerned with science as such—WMO, WHO, FAO, ITU, or UNESCO itself—but UNCTAD, whose name is becoming more and more closely linked with the current enthusiasm for the "transfer of technology"; and this, apparently, is to be the real theme for discussion in 1979.

What information is available about plans for the conference indicates that it will follow in a general way the pattern set by the Stockholm environment conference of 1972. This involves a series of pre-conferences at regional level, intended particularly to digest the now-familiar country reports from governments, and a "forum", more serious in intent than that at Stockholm, at which organisations and presumably individuals not represented at the conference itself can debate the approaches to science and technology most likely to be of use to the Third World.

Two innovations will be welcome to those who have felt that the dead hand of the UN Secretariat predisposes all such meetings to argument rather than action. One is the CSTD's insistence that the conference secretariat should be seconded from the agencies directly involved in the subjects to be discussed. The other is that the conference secretary-general should be selected by the CSTD, being the body most closely concerned, and not presented as the personal choice of the UN's Secretary General, as has hitherto appeared to be the case.

It was this last issue that occupied a whole week of participants' time in New York. At first, it seems, Third World representatives insisted that the secretary-general should be chosen from among their own eminent scientists or technologists. In this they were backed by a number of other countries, notably, Australia, Canada and Sweden. When it became apparent that there was just no suitable candidate available from within this group, it was agreed to accept one from outside.

There is, as it happens, one widely canvassed name: Guy Gresford, who was in fact Australia's representative at the meeting. As former secretary of the UN's Advisory Committee on the Application of Science and Technology, he is known and respected by governments and UN specialised agencies alike; he is acceptable to the Third World majority group; and as a quiet, confident administrator he is as likely as anyone to be able to see fair play in the battle already being joined between the various members of the UN family. They are now for the first time embarking on a major inter-agency conference; its success will depend largely on their ability to work together for the benefit of the countries whose aspirations they so often claim to support. □

Give yourself a write-up

One of the reasons that is regularly given for the decline in numbers of students wanting to study natural sciences and the concomitant growth in the numbers of those wanting to study, in particular, medicine and law, is that whilst medicine and the law is about people, science is about things and is therefore less human. Although the argument about the people-orientation of the law is one that is open to considerable debate, it is more important that the argument that science is about things is not allowed to pass unchallenged. For if science cannot present itself as a human activity, it not only loses potential bright students but it also positively encourages a science-for-people movement which at times seems only too glad to present science as practised by heartless technicians.

There have, of course, been a few who were prepared to say that science is only what its practitioners can agree amongst each other and thus does not reside ultimately in textbooks or learned journals. This puts science firmly in the realms of discussion, persuasion, criticism and dissent—very human activities. But scientists have rarely helped along this cause by leaving for others a very helpful record of their intellectual development, and if pressed have tended to regard scientific publications as adequate evidence.

At the same time, the historians of science seem to have increasingly fought shy of the twentieth century, not just because many of the major figures are still around (this, after all, has not inhibited historians in general) but also because to write on the recent past without putting it into a social, even ideological framework is somehow more difficult, although no less necessary. And social and ideological frameworks have become so much the fashion.

If there are a few very distinguished exceptions, such as Margaret Gowing both with her histories of atomic energy and her Contemporary Science Archives in Oxford, these only point to the need for more. If historians take too much refuge in Newton and sociologists in counting references, scientists should step into the breach themselves.

The web of personal relationships, ideas discarded, intellectual growth, differences of opinion in science is perhaps less fascinating to the general public than the revelations of a film-star or even a vet. But it is certainly not worthless, and it can only really be relayed at first hand—by autobiography. Many published autobiographies are deceptive, self-serving or tedious but the accumulation of them helps a clear picture to emerge over the course of the years.

Most writers of autobiography have more than simply a desire that their world-view should be widely known; they are used to making a living by writing and view an autobiography as one more way of keeping the wolf from the door. The scientist does not live under the same sort of shadow and perhaps this is one of the reasons that scientific autobiography is rare. Another may well be that the skills, in recent years, of a Waugh, Russell or Muggeridge positively inhibit non-literary characters from putting pen to paper. As a result, however, we are sadly deficient in any personal records of some of the scientific developments of the past fifty years—what people used to do, think, read, be influenced by, care about—and biography is not sufficient to remedy this (nor for that matter is scientific biography all that frequent at the sort of depth which helps one understand the human side of science).

Could not more scientists, including those with no particular eminence to their name, be persuaded to spend their last year or two in a tenured and secure post prior to retirement in getting down on paper—if only for it to sit in a university archives—what doing science was really like for them? This is something that the Royal Society could well stimulate, regardless of questions of literary merit. And who knows, there may even be a few more Watsons, Snows, Medawars out there. Well, one or two. ☐

The human breeding strategy

Paul A. Colinvaux, Professor of Zoology at Ohio State University, takes issue with what Nature *in a recent editorial called "the now widely accepted view" that high population growth is a consequence and not the cause of poverty*

The view that high population growth is a consequence and not the cause of poverty is widely held by demographers, but it is not held by ecologists interested in breeding strategies of Darwinian species. The breeding stategy of the species *Homo sapiens* is comparable to that of many birds: a few large young are produced at a time, then fed and protected for a long period. This breeding strategy, which I have called "the large young gambit", confers fitness by yielding a high return of surviving and reproducing young for each unit of resource capital invested. The advantage of being given the large young gambit by natural selection can readily be seen by contrasting it with the breeding strategies of species which make very large numbers of small eggs or seeds, what I call "the small egg gambit".

Small eggs, and the tiny young which hatch from them, are at high hazard, so that most of them die. Since every tiny corpse represents a unit of resource investment upon which there is no return, simple economic arguments show that fitness is likely to be greater when a small number of large young are reared and protected than when multitudes of tiny progeny are cast out to fend for themselves. The breeding strategy of people, therefore, is one that gives them great ability to

obituary

Gordon Miller Bourne Dobson, CBE, ScD, FRS, former reader in meteorology in the University of Oxford, died at the age of 87 on March 10. After school at Sedbergh he went up to Gonville & Caius College, Cambridge. The post he held from 1911–1913, as student-assistant at Kew Observatory, followed by the Lectureship in Meteorology at the Central Flying School at Upavon, established the broad interest in the physics of the atmosphere which dominated his long and productive life. During his period at the Royal Aircraft Establishment, Farnborough, in the First World War, he was invited by Professor F. A. Lindemann to join him at the Clarendon Laboratory, Oxford, where he was appointed University Lecturer in 1920.

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Fortunately Dobson has left us with a full and entertaining account of his own work up to 1966: Forty years' research on atmospheric ozone at Oxford: a history, *Applied Optics*, **7**, 387–405 (1968). After that date he continued making substantial contributions to the study of atmospheric ozone, involving thousands of observations on 'No. **1**', the prototype photoelectric photometer built with his own hands in 1927 and only slightly modified since (photomultiplier and electronics). His work was done almost entirely at his home, and to a great extent financed out of his own pocket. There was a regular pattern of morning work, in the study if necessary but preferably in the workshop or observing hut, gardening in the afternoon (all weathers), further work after tea and reading (mainly scientific but in his last years general and voracious) after supper. If this regime sounds monastic, he was no hermit, and visitors to Watch Hill were always welcome; after tea or morning coffee Dobson was always eager to discuss some new observations, demonstrate some new apparatus, or discuss the recent literature.

His gift of going directly to the heart of a problem made him an outstanding speaker and his enthusiasm was infectious: his lectures on the atmosphere at the Clarendon Laboratory were always packed.

In the ozone community he seemed a permanent fixture and we are still staggered by his leaving us; for those who were privileged to work closely with him there are treasured memories and deep gratitude for his life and example.

C. D. Walshaw

Karl Weissenberg was born in Vienna, Austria on 11th June 1893 and attended many different schools in Austria, Germany and France before matriculating in Frankfurt a/Main at the age of sixteen and a half. He subsequently studied at the Universities of Vienna, Berlin and Jena, taking Mathematics as his main subject, with Physics and Chemistry as subsidiaries, touching also upon Law and Medicine. He became a Privat-Dozent and, later, Professor of Physics at the University of Berlin.

In 1922 he joined the research team of M. Polanyi at the Kaiser Wilhelm Institute für Faserstoffchemie in Dahlen, Germany, and there, for a period of about six years, worked on X-ray crystallography. His fame in this field rests on his invention of a new X-ray goniometer, which allowed for the first time, a unique determination of 3-dimensional crystallographic structure. The idea was only slowly adopted, and it was not until after the war that the Weissenberg X-ray Goniometer, as it is known, came into general use. By 1929 his attention turned from crystallography to rheology and he presented a general analytical method for finding the shear rate at a capillary wall, now widely used.

Just before the war, Weisenberg took up residence in Britain, and his wartime work on flame thrower fuels stimulated his curiosity and interest in the so-called anomalous liquids, and hence in the field of liquid rheology itself. He also demonstrated, by means of rubber and fabric sheets, the importance of rotation of the embedded co-ordinate system in a flowing continuum. He had much earlier conjectured that certain fluids were elastic and now came the inference of the existence of a tension along the streamlines, since amply demonstrated. At this time, too, the essence of the Weissenberg Rheogoniometer was established. The principle behind this second major invention was that it allowed the stresses around the full solid angle in a flowing continuum to be experimentally determined.

In addition to a deep and sensitive appreciation of a range of natural phenomena, Karl Weissenberg was an entirely engaging and unselfish person. His accord with nature was faithfully mirrored in that with his fellow human beings. He was notable in his scientific achievements and noble in personal qualities.

John Harris

announcements

Awards

The American Society for Microbiology has awarded:

The **Wyeth Award** in Clinical Microbiology to **Edwin H. Lennette** of the California Department of Health.

The **Eli Lilly and Company Award** to **Ronald W. Davies** of Stanford University School of Medicine.

The **Carski Foundation Distinguished Teaching Award** to **Elizabeth R. Hall** of Washington State University.

The Biochemical Society has given:

The **BDH Award in Analytical Biochemistry** to Professor John Landon of St Bartholomew's Hospital.

The **Colworth Medal** to Dr W. J. Brammar of Edinburgh University.

The **Keilin Medal** to Dr S. M. E. Magnusson of the University of Aarhus.

Meetings

July 7–9, **Annual Meeting of the Biochemical Society,** London (The Meetings Officer, The Biochemical Society, 7 Warwick Court, High Holborn, London WC1R 5DP).

June 23, **Nuclear Medicine Imaging Systems,** London (British Institute of Radiology, 32 Welbeck Street, London W1M 7PG).

October 12–15, **Evolution and Lateralisation of the Brain,** New York (Conference Department, the New York Academy of Sciences, 2 East 63rd Street, New York 10021).

August 30–September 3, **The Chemistry and Uses of Molybdenum,** Oxford (Molybdenum Conference, Climax Molybdenum Co. Ltd., Villiers House, 41/47 Strand, London WC2N 5JS, UK).

September 21–23, **Autumn Meeting,** Sheffield (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN).

Reports and publications

Great Britain

Forestry Commission. Forest Mensuration Handbook. (Forestry Commission Booklet No. 39.) By G. J. Hamilton. Pp. 274. (London: Her Majesty's Stationery Office, 1975.) £4.00 net. [162

HMSO Subject Catalogue No. 1. Statistics. (Publications of UK Government Departments and International Organisations. Pp. iii + 55. (London: HMSO 1975.) [172

Universities Research Reactor. Annual Report, 1974–75. Pp. iii + 36. (Warrington: Universities Research Reactor, Risley, Warrington, 1976.) [172

University of Glasgow. The Hannah Research Institute: for Studies Relating to the Production and Utilization of Milk, Report 1975. Pp. 71. (Ayr: The Hannah Research Institute, 1976.) £1.00. [192

The Year Book of the Royal Society of London 1976. Pp. 425. (No. 80). (London: The Royal Society, 1976.) £3.20. [232

Department of the Environment. Sixteenth Progress Report of the Standing Technical Committee on Synthetic Detergents. Pp. v + 20. (London: HMSO, 1976.) 65p net. [242

Royal Observatory, Edinburgh. Annual Report, 1974/1975. Pp. 29. (Edinburgh: Royal Observatory, 1976.) [242

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 273, No. 926: The Postcranial Skeletons of the Triassic Mammals *Eozostrodon*, *Megazostrodon* and *Erythrotherium*. By F. A. K. Jenkins and F. R. Parrington. Pp. 387–431 + Plates 1–6. UK £3.45; Overseas £3.55. Vol. 273, No. 927: A Discussion on Water Relations of Plants. By J. E. Monteith and P. W. Weatherley. Pp. 433–618. UK £9.75; Overseas £10. (London: The Royal Society, 1976.) [262

Agricultural Development Advisory Service—Ministry of Agriculture, Fisheries and Food, Wildlife Conservation in Semi-Natural Habitats on Farms: A Survey of Farmer Attitudes and Intentions in England and Wales. Pp. viii + 69. (London: HMSO, (1976.) £1.25 net. [262

Scottish Development Department. Towards Cleaner Water 1975—Report of a Second Rivers Pollution Survey of Scotland. Pp. 72 + maps A–G. (Edinburgh and London: HMSO, 1976.) £5.90 net. [262

British Antarctic Survey. Scientific Reports. No. 54: The James Ross Island Volcanic Group of North-East Graham Land. By Dr P. H. H. Nelson. Pp. 62 + 11 plates. £6 net. No. 90: Measurements of Atmospheric Ozone at the Argentine Islands and Halley Nay, 1957–72. By J. C. Farman and R. A. Hamilton. Pp. 40. £2.90 net. (Cambridge: British Antarctic Survey, 1975.) [272

Other countries

Energy: The Case for Conservation. By Denis Hayes. (Worldwatch Paper No. 4.) Pp. 77. (Washington, DC: Worldwatch Institute, 1776 Massachusetts Avenue, N.W., 1976.) $2. [22

Republic of the Sudan. Ministry of Agriculture—Agricultural Research Division. Annual Report of Yambio Research Station, 1963/1964. Pp. 19. (Wad Medani: Gezira Research Station, 1975.) [32

CERN—European Organization for Nuclear Research. CERN 75–20: The Design of the Control System for the SPS. By M. C. Crowley-Milling. Pp. 35. (Geneva: CERN, 1975.) [42

Royal College of Forestry, Stockholm. Studia Forestalia Suecica. No. 127: A Comparative Study of Chloroplast Morphogenesis in Seedlings of Some Conifers (*Parix decidua*, *Pinus sylvestris* and *Picea abies*). By Bjorn Walles and Jan Hudak. Pp. 21. No. 128: Influence of Photo- and Thermoperiod on the Initial Stages of Frost Hardening and Dehardening of Phytotron-Grown Seedlings of Scots Pine (*Pinus silvestris* L.) and Norway Spruce (*Picea abies* (L). Karst.) By Aron Aronsson. Pp. 20. No. 129: Estimating the Accuracy of Site Index Curves by Means of Simulation. By Bjorn Hagglund. Pp. 34. (Stockholm: Royal College of Forestry, 1975.) [62

Energy, Mines and Resources Canada. Geological Survey of Canada. Paper 76–1A: Report of Activities, Part A. Pp. ix + 522. (Ottawa: Information Canada, 1976.) $6. [62

Energy from Thermonuclear Fusion. Pp. 48. (Garching bei Munchen: Max-Planck-Institut fur Plasmaphysik, 1976.) [92

Some Aziridines, *N*–, *S*– & *O*–Mustards and Selenium. (IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 9.) Pp. 268 (Lyon: International Agency for Research on Cancer, 1975 Distributed by World Health Organization, Geneva and HMSO, London). Sw.fr.27. [92

United States Department of the Interior: Geological Survey. Bulletin 1388: Influence of Rainfall and Ancient Landslide Deposits on Recent Landslides (1950–71) In Urban Areas of Contra Costa County, California. By T. H. Nilsen and B. Turner. Pp. iii + 18 + plates 1 and 2. Professional Paper 774–C: Mount Abbot Quadrangle, Central Sierra Nevada, California—Analytic Data. By John P. Lockwood. Pp. iii + 18. (Washington, DC: US Government Printing Office, 1975.) [102

Biological Peroxidation of Lipids and Membranes. By M. Wolman. (Supplement to Vol. 11, *Israel Journal of Medical Sciences.*) Pp. 248. (Jerusalem: *Israel Journal of Medical Sciences*, 1975. [122

Geophysical Institute, University of Alaska. Annual Report 1974/1975. Pp. 183. (Fairbanks, Alaska: Geophysical Institute, The University, 1975.) [122

United States Department of the Interior: Geological Survey. Professional Paper 726–E: Gravity and Aeromagnetic Study of Part of the Yakima River Basin, Washington. By S. L. Robbins, R. J. Burt and D. O. Gregg. Pp. iii + 7 + plate 1. Professional Paper 743–D: Dimorphism in Two New Genera of Devonian Tabulate Corals. By William A. Oliver, Jr. Pp. iii + 11 + plates 1–7. (Washington, DC: US Government Printing Office, 1975.) [122

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 246: Sedimentology of the Archean Yellowknife Supergroup at Yellowknife, District of Mackenzie. By John B. Henderson. Pp. ix + 62 $6. Bulletin 247: Triassic Rocks of the Rocky Mountain Foothills and Front Ranges of Northeastern British Columbia and West-Central Alberta. By D. W. Gibson. Pp. 61 (9 plates). $5. Bulletin 251: Investigations of Lower Paleozoic Geology, Foxe Basin, Northeastern Melville Peninsula, and Parts of Northwestern and Central Baffin Island. By H. P. Trettin. Pp. 177. $10. (Ottawa: Information Canada, 1975.) [122

United States Department of the Interior: Geological Survey. Professional Paper 729–D: Geology of Sedimentary Rocks in Southern Yellowstone National Park, Wyoming. (Geology of Yellowstone National Park.) By J. D. Love and W. R. Keefer. Pp. x + 60. (Washington, DC: US Government Printing Office, 1975.) [162

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US Department of Health, Education and Welfare. Health Manpower in the Changing Australian Health Services Scene. By Ruth Roemer and Milton J. Roemer. Pp. viii + 87. (Springfield, Virginia: National Technical Information Service.) [172

Environment Canada: Fisheries and Marine Service. Technical Report No. 584. Intestinal Trematode Parasites (Trematoda: Digenea) and Food of Yellowtail Flounder (*Limanda ferruginea* (Storer 1839)) from the Scotian Shelf and Gulf of St. Lawrence. By J. S. Scott. Pp. iii + 12. Technical Report No. 593. Diogenetic Trematode Parasites and Food of Witch Flounder (*Glyptocephalus cynoglossus* L.) from the Scotian Shelf and Gulf of St. Lawrence. By J. S. Scott. Pp. iv + 9. Technical Report No. 594. Catch Statistics for the Bay of Fundy Herring Fisheries 1963–1974. By D. S. Miller and T. D. Iles. Pp. iii + 75. (St. Andrews, N.B.: Department of the Environment Fisheries and Marine Service, 1975.) [182

Smithsonian Contributions to Zoology, No. 203. New Species of Marine Molluscs from Pitcairn Island and the Marquesas. By Harald A. Rehder and Barry R. Wilson. Pp. iv + 16. (Washington, D.C.: Smithsonian Institution Press, 1975. For Sale By US Government Printing Office.) [182

Department of Energy Mines and Resources: Geological Survey of Canada. Paper 74–47. Upper Paleozoic Rocks of the Atlin Terrane, Northwestern British Columbia and South Central Yukon. By J. W. H. Monger. Pp. vi + 63 + 5 maps. (Ottawa: Information Canada 1975.) $3.50; other countries $4.20. [202

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National Research Council Canada. Associate Committee on Scientific Criteria for Environmental Quality—Status Report. Pp. 68. NRCC No. 14109: Waste Heat in the Aquatic Environment. By D. R. Dickson. Pp. 39. $1. (Ottawa: Publications, National Research Council Canada, 1975.) [232

Mitteilungen aus der Biologischen Bundesanstalt fur- Land und Forstwirtschaft, Berlin-Dahlem. Heft, 166: Resistenzbildung gegen systemische Fungizide (Benzimidazolderivate) bei Colletotrichum Lindemuthianum (Sacc. et Magn.) Bri. et Cav. Von Dr Ehler Meyer. Pp. 135. (Berlin-Dahlem: Biologisches Bundesanstalt fur Land- und Forstwirtschaft, 1976.) DM 16.60. [242

Person to Person

Wanted: A copy (preferably hardback) of 'The Discovery of Time' by Toulmin and Goodfield (Hutchinson, 1965). Please contact Dr Peter J. Smith, Department of Earth Sciences, The Open University, Walton, Milton Keynes MK7 6AA, England, giving price, condition, etc.

The New York Academy of Sciences seeks, by July 1, nominations for its annual awards from individuals or organisations. For futher information write to: The Chairman of the awards committee, The New York Academy of Sciences, 2 East 63rd Street, New York, New York 10021.

A symposium entitled, 'Pesticide Induced Delayed Neurotoxicity', jointly sponsored by the US Environmental Protection Agency and the US National Institute of Environmental Health Sciences was held in Washington, DC, February 19–20, 1976. The full text of the papers and ensuing discussions of the basic scientific and regulatory aspects of organophosphate-induced delayed neurotoxicity are to be made available free to interested individuals and institutions. All inquiries should be addressed to Dr R. L. Baron, Health Effects Research Laboratory, Environmental Protection Agency, Research Triangle Park, North Carolina 27711.

The André Lightwitz prize (8,800 francs) is awarded to French or foreign scientists who have specially distinguished themselves in research on calcium and phosphorus metabolism, whether in the clinical or more fundamental fields. Conditions of entry for the 1976 prize can be obtained from INSERM. Applications must reach the Director of the INSERM (c/o Dr Lucienne Bonnot, 101 rue de Tolbiac, 75645 Paris cedex 13, France) by June 30, 1976.

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

nature June 3, 1976

UN prepares for the next round

It is a safe bet at this time of Habitat that few people, even those concerned with United Nations affairs, have ever heard of the CSTD. The acronym stands for the Committee on Science and Technology for Development. At present, unlike many such international groups, the CSTD provides a forum for debate, rather than the sort of political battlefield which is now all too familiar. But the protagonists are the same: on the one side the countries of the Third World, on the other the industrialised, affluent countries. At the CTSD's third meeting, in New York earlier this year, the debate was concentrated for something like half the 3-week session on a single issue: who should run the United Nations Conference on the Application of Science and Technology to Development, now scheduled to take place, probably in Vienna, in 1979.

Even the proposal for this event has received hardly any publicity, inside as well as outside the UN; but enough information is now available to indicate certain differences from previous gatherings in the long series that started, in effect, with the first Atoms for Peace Conference in 1955. Probably the most important point is that from the start this has been intended as an inter-agency conference, the UN itself playing a central but much smaller role than hitherto. This should have the immediate advantage that the specialised agencies, almost all of which have a stake somewhere in the field of applied science or technology, need not feel that the meeting will be dominated by the political preoccupations of the UN.

At the same time, it is impossible to envisage the preparation for such a conference without continual and probably bitter in-fighting between the specialised agencies themselves—as witness the unheralded personal appearance of Mr M'Bow, the Director General of UNESCO, to ensure that his agency obtained a fair hearing at the recent session in New York. Probably one reason for this was that the organisation principally interested in the conference appears to be not one of those concerned with science as such—WMO, WHO, FAO, ITU, or UNESCO itself—but UNCTAD, whose name is becoming more and more closely linked with the current enthusiasm for the "transfer of technology"; and this, apparently, is to be the real theme for discussion in 1979.

What information is available about plans for the conference indicates that it will follow in a general way the pattern set by the Stockholm environment conference of 1972. This involves a series of pre-conferences at regional level, intended particularly to digest the now-familiar country reports from governments, and a "forum", more serious in intent than that at Stockholm, at which organisations and presumably individuals not represented at the conference itself can debate the approaches to science and technology most likely to be of use to the Third World.

Two innovations will be welcome to those who have felt that the dead hand of the UN Secretariat predisposes all such meetings to argument rather than action. One is the CSTD's insistence that the conference secretariat should be seconded from the agencies directly involved in the subjects to be discussed. The other is that the conference secretary-general should be selected by the CSTD, being the body most closely concerned, and not presented as the personal choice of the UN's Secretary General, as has hitherto appeared to be the case.

It was this last issue that occupied a whole week of participants' time in New York. At first, it seems, Third World representatives insisted that the secretary-general should be chosen from among their own eminent scientists or technologists. In this they were backed by a number of other countries, notably, Australia, Canada and Sweden. When it became apparent that there was just no suitable candidate available from within this group, it was agreed to accept one from outside.

There is, as it happens, one widely canvassed name: Guy Gresford, who was in fact Australia's representative at the meeting. As former secretary of the UN's Advisory Committee on the Application of Science and Technology, he is known and respected by governments and UN specialised agencies alike; he is acceptable to the Third World majority group; and as a quiet, confident administrator he is as likely as anyone to be able to see fair play in the battle already being joined between the various members of the UN family. They are now for the first time embarking on a major inter-agency conference; its success will depend largely on their ability to work together for the benefit of the countries whose aspirations they so often claim to support. ☐

Give yourself a write-up

One of the reasons that is regularly given for the decline in numbers of students wanting to study natural sciences and the concomitant growth in the numbers of those wanting to study, in particular, medicine and law, is that whilst medicine and the law is about people, science is about things and is therefore less human. Although the argument about the people-orientation of the law is one that is open to considerable debate, it is more important that the argument that science is about things is not allowed to pass unchallenged. For if science cannot present itself as a human activity, it not only loses potential bright students but it also positively encourages a science-for-people movement which at times seems only too glad to present science as practised by heartless technicians.

There have, of course, been a few who were prepared to say that science is only what its practitioners can agree amongst each other and thus does not reside ultimately in textbooks or learned journals. This puts science firmly in the realms of discussion, persuasion, criticism and dissent—very human activities. But scientists have rarely helped along this cause by leaving for others a very helpful record of their intellectual development, and if pressed have tended to regard scientific publications as adequate evidence.

At the same time, the historians of science seem to have increasingly fought shy of the twentieth century, not just because many of the major figures are still around (this, after all, has not inhibited historians in general) but also because to write on the recent past without putting it into a social, even ideological framework is somehow more difficult, although no less necessary. And social and ideological frameworks have become so much the fashion.

If there are a few very distinguished exceptions, such as Margaret Gowing both with her histories of atomic energy and her Contemporary Science Archives in Oxford, these only point to the need for more. If historians take too much refuge in Newton and sociologists in counting references, scientists should step into the breach themselves.

The web of personal relationships, ideas discarded, intellectual growth, differences of opinion in science is perhaps less fascinating to the general public than the revelations of a film-star or even a vet. But it is certainly not worthless, and it can only really be relayed at first hand—by autobiography. Many published autobiographies are deceptive, self-serving or tedious but the accumulation of them helps a clear picture to emerge over the course of the years.

Most writers of autobiography have more than simply a desire that their world-view should be widely known; they are used to making a living by writing and view an autobiography as one more way of keeping the wolf from the door. The scientist does not live under the same sort of shadow and perhaps this is one of the reasons that scientific autobiography is rare. Another may well be that the skills, in recent years, of a Waugh, Russell or Muggeridge positively inhibit non-literary characters from putting pen to paper. As a result, however, we are sadly deficient in any personal records of some of the scientific developments of the past fifty years—what people used to do, think, read, be influenced by, care about—and biography is not sufficient to remedy this (nor for that matter is scientific biography all that frequent at the sort of depth which helps one understand the human side of science).

Could not more scientists, including those with no particular eminence to their name, be persuaded to spend their last year or two in a tenured and secure post prior to retirement in getting down on paper—if only for it to sit in a university archives—what doing science was really like for them? This is something that the Royal Society could well stimulate, regardless of questions of literary merit. And who knows, there may even be a few more Watsons, Snows, Medawars out there. Well, one or two. ☐

The human breeding strategy

Paul A. Colinvaux, Professor of Zoology at Ohio State University, takes issue with what Nature *in a recent editorial called "the now widely accepted view" that high population growth is a consequence and not the cause of poverty*

The view that high population growth is a consequence and not the cause of poverty is widely held by demographers, but it is not held by ecologists interested in breeding strategies of Darwinian species. The breeding stategy of the species *Homo sapiens* is comparable to that of many birds: a few large young are produced at a time, then fed and protected for a long period. This breeding strategy, which I have called "the large young gambit", confers fitness by yielding a high return of surviving and reproducing young for each unit of resource capital invested. The advantage of being given the large young gambit by natural selection can readily be seen by contrasting it with the breeding strategies of species which make very large numbers of small eggs or seeds, what I call "the small egg gambit".

Small eggs, and the tiny young which hatch from them, are at high hazard, so that most of them die. Since every tiny corpse represents a unit of resource investment upon which there is no return, simple economic arguments show that fitness is likely to be greater when a small number of large young are reared and protected than when multitudes of tiny progeny are cast out to fend for themselves. The breeding strategy of people, therefore, is one that gives them great ability to maximise the return, particularly in circumstances of limited resources. It is a property they have in common with all followers of the large young gambit, with sparrows, antelopes, viviparous snakes, and great white sharks.

David Lack showed us 20 years ago that an essential requirement of the large young gambit is that the size of the family be closely gauged to the available resources. He produced good evidence that the clutch size of many birds is dependent on the food supply, the number of eggs laid varying from season to season. The original data can be found in his *Natural Regulation of Animal Numbers*, and there has since been overwhelming confirmation of his work. The mechanism deciding the clutch-size for a species following this gambit must ensure that the chosen clutch is not slightly too small for the available resources, or the genes for this mechanism will be removed from the population by natural selection favouring those genotypes which gave their possessors a slightly more ambitious clutch. But it must also ensure that the clutch is not too large, because an attempt to rear a family too numerous for the available resources puts all the progeny at hazard. The most fit individuals will be those which assess the food and other resources of their living space with the most precision.

Natural selection was undoubtedly alive and well when the breeding strategy of the Pleistocene populations of *Homo* was fashioned. Our ancestors were, therefore, adapted to make the best possible choice for the number of children in a family, "best" meaning the largest number for which there was a high probability of it being possible to rear them to maturity. There can be little doubt that the workings of intelligence gave people a skill in applying this breeding strategy which could not be equalled by birds, sharks, and the rest. It is against these Darwinian realities that there is scope for reappraisal of what we know of reproductive habit and taboo in primeval societies. Infanticide, for instance, appears to be, not a device for impeding population growth, but rather a device for increasing it. Infanticide is one of the mechanisms provided to human families by natural selection not to make the family too large for the available resources. It increases fitness. The pill, the diaphragm, and the lump of plastic perched in the uterus must be expected to work in the same way. Contraceptives ought to increase Darwinian fitness—a thought for those who ponder the failure of birth control schemes.

It appears that no human population has abandoned the Darwinian breeding strategy of our Pleistocene ancestors. Every couple continues to choose the largest number of children it can afford to rear with a high expectation of success. The change we have made is to abandon many parameters of the old niche, replacing them with surrogates, and we have added to the niche many new parameters or alternatives. These changes in niche are well understood: farming instead of gathering; herding instead of hunting; concentrating in cities, which allows "agribusiness" and the bringing of food to people instead of people to food. These changes in niche are the real measure of the difference between people and the rest of Darwinian species, none of which can change their ways of life to suit their convenience as we can.

If a niche requires many resources, or allows many alternatives, an ecologist calls it "broad"; if a species is of stereotyped habit and highly specialised, its niche is called "narrow". Humanitarians refer to similar things when they talk of "riches" and "poverty". Rich people have large niches, poor people have small ones. It takes more resources (tangible and intangible) to provide niche space for a rich human couple than for a poor one.

But rich and poor alike retain their large-young breeding gambit. Each couple of both assesses the resources of its habitat in ways which have been programmed for them by natural selection from the times of remotest ancestors. We are conscious of these ways when we know that we love children. And our process of resource-assessment must always have taken into account the traditional ways of life. Even primeval people learned how to live from their parents and their parents' peers. Darwinian fitness (given by success at child-rearing) for these people must have been dependent on providing for each child the resources needed to let it grow up to live in the traditional way, for otherwise it would never be equipped to mate and rear children itself. A couple planning a family estimated, as it still does, the resources needed to raise a child to live as well as the parents lived. The number of children the couple chooses is the largest for which traditional resources can be provided. That people still behave so is the explanation for the fact that families in affluent countries tend to be smaller than families in poor countries, the so-called demographic transition. A well-off couple chooses the most children it can raise to well-off standards or even to slightly better standards, which is not likely to be many. The poor only have to provide for each child what is needed to raise it in poverty, which may allow for more children even when the actual resource base is less.

It is our experience with the demographic transition which has allowed the claim to be made that high population growth is a consequence and not a cause of poverty. The element of truth which lies in the claim is the observed fact that families of the affluent tend to be smaller, for the good Darwinian reasons described above. But this is not to say that "high" population growth does not happen in the affluent (it can and does—see the history of the United States); it is merely to claim that the highest growth rates of the contemporary world are in poor nations. And it speaks not at all to the question of the cause of poverty itself. This too can be understood and predicted from a knowledge of the human habit of changing niche while keeping breeding strategy constant.

While people retain their excellent large-young gambit they can be expected to maximise the return, and this is true regardless of their standards of life. Increase in population with time will have a slightly higher value for the poor, but this difference in value makes no difference to the size of the eventual population limit. Poverty is, of course, a relative value, and is better replaced in the interests of clear thinking by some measure of resources, or by the concept of niche-size itself, which G. E. Hutchinson showed us could in theory be quantified (see *Cold Spring Harbor Symposium on Quantitative Biology*). If riches and poverty are large niches and small, then they are properties of the size of population at all times except during rapid population growth.

Times of rapid growth are the exception because these must be times of excess resources. This is the explanation of affluence and rising numbers going together in the United States during the last century. There were for a time so many spare resources that almost unlimited choices for life were possible, giving rise to the most extravagant portions of the American Dream. But as numbers close on resources, as the population presses on the population limit, choice must go. Then, if the numbers continue to rise, the resource allocation per individual (its niche space) must decrease. People can accept changed niches, it is their unique quality. They continue to maximise the return even as the upper limit for their traditional way of life is reached. The next generation must respond by reducing the size of the niche so that the limit can be increased. But they, in turn, still maximise the return. All poverty is caused, in the long run, by the continued growth of population. The continued growth of population itself is caused by the combination of our excellent Darwinian breeding strategy and our ability to change the niche within wide limits. ☐

Iranian transplant

Iran is in a hurry to industrialise, and needs teachers and technicians. **Peter Newmark,** just back from Tehran, assesses the problems

EVEN a brief visit is enough to show that the development of science and technology in Iran is hurtling forward, somewhat blindly, along the paths well worn by the Western countries. Using but a tiny portion of his oil revenues, the Shah (or, more properly, the Shahanshah or King of Kings) has instigated an immense programme of higher education to produce the technologists and scientists that his country needs to hoist it from "developing" to "developed" status. But all the oil in Persia cannot make the hoisting operation run smoothly.

In other circumstances a country with an illiteracy rate of 50% would focus its attention on a programme of general education at the initial expense of higher education. Iran, however, is in a hurry, and under few enough financial restraints that it can undertake parallel improvements at all levels of learning. The Shah has therefore recently introduced free compulsory education to the age of 13 and, last year, added free university education in return for a commitment to join the civil service for a period of twice the length of the degree course.

The consequent demand for higher education has greatly exceeded predictions. This year there are eight applications for every university place that is available, in spite of the fact that the number of places has doubled in less than five years. In part the additional demand will be met by the introduction this year of a Free University on the radio and television networks (based on and bought from Britain's Open University).

The clamour for higher education is a consequence of over-eager policies, particularly in a country where the the material advantages that accrue to the highly educated minority can all too clearly be seen. The problem is both to meet the demand and to channel it sensibly. In the first place there is a drastic need to reinvest many of the educated into the teaching system, which at present suffers from manpower shortages in many areas.

The other priority must be to solve Iran's shortage of skilled technicians. That problem is the classic one faced by any country that suddenly finds itself able to buy up the highly advanced technology of the developed countries—be it a nuclear power plant or an electron microscope—but without the trained technicians needed to run and maintain the equipment. For example, Dr Zarghami, Chancellor of the Arya-Mehr University of Technology in Tehran, estimates that the country presently lacks 2,000 of the 28,000 graduate engineers that it requires. But worse is to come. In ten years time he predicts that Iran will only be able to provide 60,000 of the 75,000 graduate engineers needed.

That problem is essentially insoluble in the short term. It is not even clear that the output of graduate engineers can really be more than doubled in ten years despite the founding of a second campus of the University of Arya-Mehr. The campus, a self-contained city, will be situated a few miles outside the city of Isfahan, 200 miles south of Tehran. When fully functional the Isfahan campus will be the largest technical university in the Middle East with the possible exception of the Technion in Haifa. In ten years time it will have 10,000 undergraduates and 3,000 graduate students.

Another part of the plan to increase the number of technicians is the intention of the Ministry of Science and Higher Education to collaborate with Iranian industry in setting up an Industrial Training Complex. Its sole function would be to turn out specialist technicians to satisfy the requirements of industry. At a lower level there is already a requirement that any factory which employs more than 500 people has attached to it a school for the children of the factory workers. Such schools can instruct the children in at least some of the basic skills required for them to follow their parents into the factory.

In spite of all these plans the shortage of specialist technicians will still be increasing rather than decreasing for at least the next ten years. The gap will have to be met by offering high salaries to skilled personnel either from the West or from other Middle East countries. Recruitment from the latter, to judge from accounts of the recent Islamic Solidarity Conference in Science and Technology, is bound to provoke regional discontent.

With such a shortage of teachers and technicians it would not be surprising were the progress of basic scientific research to be in jeopardy. On the contrary, however, a clear and strong commitment to the establishment of basic research is evident both in universities and in separate research institutes in Iran, although fears are expressed in some quarters that the level of future support will depend very directly on the sales of oil which, together with the revenue thereby derived, fell sharply last year.

Cam & Pen Int.

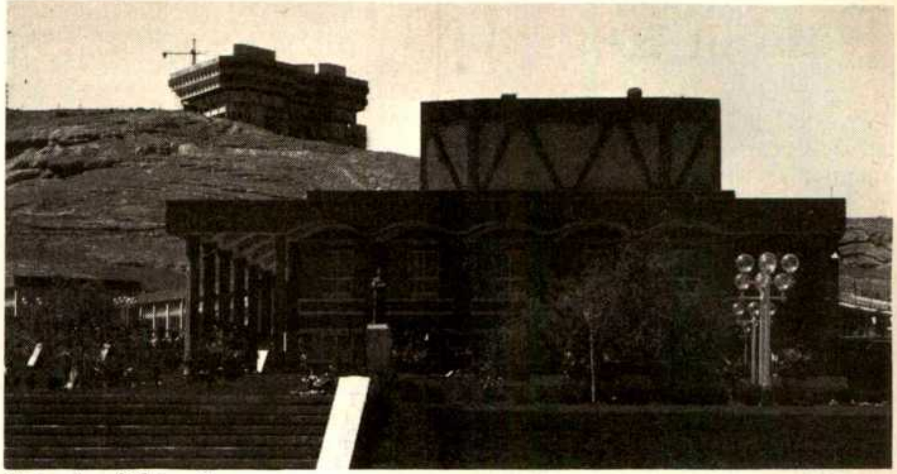
Iranian village

In the meantime the administration of research is very much along Western lines. For example, last year saw the creation of the National Scientific Research Council, a central policy body. The council is responsible for the coordination, supervision and funding of research. The latter will be subject to the usual kind of peer group approval and will take into account the council's suggestion of priority areas for research. At the moment, however, even Dr Parsa, Deputy Minister for Science and Research, admits that priorities are largely hypothetical since any scientist who is of reasonably high calibre (and who is at least not openly critical of the Shah's policies) is likely on those grounds alone to funded. That situation is likely to remain for some considerable time until the number of good research scientists reaches a critical mass. Demand will then no doubt start to outstrip the supply of funds.

Until (and to ensure that) the critical mass is reached, it is essential that Iranian scientists undergo part of their training overseas and that international contacts are lovingly fostered. At present about 50,000 Iranians are overseas partaking of some form of higher education. Many science graduates like to obtain their PhD overseas, but Dr Mofidi, the Vice-Chancellor of Tehran University, would prefer to see overseas training at a post-doctoral level so that it can be based on a genuine familiarity with what awaits the scientist on his or her return. And return he or she almost certainly will, given the very favourable job market in Iran compared to the West. That may also enable Iran to buy up a number of Western scientists to tide them over the next few years.

International contacts are necessary not only for the placing of students oveaseas but also for the development of research programmes. Contacts are encouraged through sabbatical leave every fourth year and by financing international meetings in Iran. A recent example was the four-day meeting on the eukaryotic genome at the University of Tehran in May. About 40 Western experts were imported to expound on their research before an audience of about the same number of local scientists. No doubt the prestige of such an occasion is as important as the chance of making contacts; the actual symposium presentation probably come a poor third in real value.

Whereas Iran is presently looking determinedly to the West for international collaboration and advice, many Iranian scientists would like to develop a more regional approach. In particular that would make sense for programmes of "regional technology" in, for example, solar energy, desalination and arid zone research. Already there are programmes of cooperation with various Arab countries, Turkey and Pakistan. A bigger attraction for some would be Israel because of her scientific expertise both in basic research and in "regional technology". Although there is already a certain amount of informal contact with Israel, political factors rule out any real cooperation.


University of Shiraz, Iran

There is in Iran an ancient tradition of science and technology. Its origins lie in the precise requirements of the Islamic faith for the time and direction of prayer. But tradition has nothing to offer to the new generation of Iranian scientists who are whole-heartedly committed to transplanting Western science, warts and all, to the Middle East. Whilst recognizing the need for a scientific discipline to have roots in the society that supports it, they argue that Iranian society is in the process of general change and in time will be ready to nourish the roots of Western-style science. The concurrent argument is that without generous support of basic research, no roots, in the form of Iranian eminence, will form.

The transplantation of Western values in science and much besides has, not surprisingly, led to certain problems in the universities as well as in Iranian society at large. In particular there are difficulties of adjustment for the growing number of students who come from poorer, more traditional backgrounds. The result is a high drop-out rate and sporadic political dissent which is ruthlessly suppressed. Overall, the Shah will not for the present tolerate any public questioning of his drive to make Iran a military and industrial force to be reckoned with. ☐

Conservation in Iran

Peter D. Moore, recently in Iran, looks at attempts to conserve wildlife in a fast changing country

NATURE conservation in the industrialised nations is said to be a cause associated primarily with the middle classes. Developing countries often see it as a worthwhile economic exercise, since wildlife parks can be a major tourist attraction providing foreign currency. But in Iran it is neither of these. There is little tourist industry apart from that which centres upon the archaeological rather than wildlife potential of the country. Yet conservation ranks high among the priorities of the government. The Iranian Department of the Environment, headed by Eskandar Firouz, is (unlike the British establishment of the same name) primarily concerned with environmental research and practical management problems in Iran's cities, rural areas, wildlife parks and reserves, and around its Persian Gulf and Caspian Sea coasts.

The sheer size of the country (about three times the area of France) and its altitudinal variation (from 50 m below sea level at the Caspian to 5,700 m in the Elburz mountains near Tehran) results in a wide range of habitats and a high diversity of wildlife. About 80% of Iran is over 1000 m in altitude: much of this lies in a great central plateau surrounded by high mountains, the physical isolation of which could account for the high proportion of endemics in the Iranian flora.

To ensure that examples of all the major habitats are conserved, the Environment Department has established over 40 wildlife parks and protected areas, covering a combined area larger than Switzerland. The broad-leaved forests of the Caspian area still contain wild pig, brown bear and the Persian subspecies of fallow deer, which has been rescued from the verge of extinction. Tigers were reported from this region in the first half of the century, but are now feared extinct.

The Caspian lowlands also contain some important wetland areas which serve as winter feeding grounds for large populations of wildfowl that breed in western Siberia. Other wetland areas in Iran are mainly in the west, especially the saline lakes: the largest is Lake Rezaiyeh, which extends over 3,000 ha and supports breeding populations of 25,000 pairs of greater flamingo and 1,400 pairs of white pelican. The wetlands of the Gulf in the south of Iran include the Eastern Mesopotamian marshes and the rivers of Iranian Baluchistan, where the marsh crocodile still survives at the western limit of its range.

The wealth of wetland habitats which Iran contains, coupled with the threat which is posed to them by increasing industrialisation, may explain why Iran has become a global trend-setter in the field of wetland conservation. In February 1971, a Convention was agreed at an international conference in Ramsar, Iran, by which all signatory member countries should designate at least one of their wetland sites for inclusion in a list of wetlands of international importance. Iran was the second country to ratify this treaty, while it has taken the British bureaucratic system five years to achieve this formality, despite the lip-service paid to conservation.

Probably the greatest threat to Iranian wetlands is posed by the industrial pollution of the Caspian Sea and the oil pollution of the Persian Gulf. In the Caspian much of the pollution is derived from the Soviet Union, but there is now cooperation between the two countries in trying to reduce its level. Worst affected is the fishing industry, where the production of sturgeon and caviar provides an important export industry. Attempts to reduce pollution are being coupled with the building of fish breeding stations. Near Rasht on the Sefid river, which runs into the Caspian, one such site of 132 ha has been built with Soviet aid and at a cost of $5 million. It produces 4 million fish per year which are released into the river.

At the other end of the habitat spectrum, almost half of the surface area of Iran is covered by desert or arid steppe, and obviously this type of habitat is well represented among its reserves. Here, in particular, human exploitation of the environment and wildlife conservation reaches a delicate and precarious balance. A relatively small percentage of Iran's population is involved in pastoralism and these people are found mainly in the semi-arid areas. Precise and accurate figures are difficult to obtain, but it has been estimated that about 400,000 pastoralists maintain a total of 30 million sheep and 15 million goats, so the grazing pressure over the arid lands is extremely high. These herds reduce the wildlife potential of the areas they graze, but it is difficult to establish whether this is a result of their consumption of herbage or simply the disturbance caused by a human presence.

The arid areas contain a rich and varied assortment of wildlife, now much depleted through hunting. Wild ass, goitered and jebeer gazelle, cheetah, leopard, wild sheep, great and houbara bustards, all still survive in viable populations within the protected regions. The large raptorial birds, particularly the golden eagle, are surprisingly frequent given that such species have often suffered at the hands of pastoralists. The maintenance of this wildlife must depend, however, upon restricting any further human pressures upon their dwindling populations, and this raises a number of social and political problems.

Before any such problems can be solved, it must be ascertained precisely what form man's interaction with wildlife takes. Studies of the productivity of the natural flora are needed urgently, particularly in the *Zygophyllum*/*Artemisia* scrub which occupies extensive tracts of the semi-desert areas. The feeding preferences and the potential consumption of the wild and domestic herbivores must also be known, so that competitive interactions can be identified and the carrying capacity of the environment for each species determined. Also, in spite of Iran's wealth of fossil fuel deposits, there is an acute shortage of fuel in the settlements of the desert area and much shrub material is gathered for combustion. A knowledge of the social practices of the people is therefore needed, including an understanding of the movements of the small groups of pastoralists who indulge in transhumance, leaving the desert plateau for the mountain pastures each spring. The historical and archaeological perspective is also important, for some desert areas have been denuded of scrub, and perhaps even of pistachio woodland, for charcoal burning and smelting in the past. It is possible that the great salt lakes or *kavirs* will contain preserved, stratified pollen from which past changes in vegetation can be determined.

An integrated study of these various aspects of the desert ecosystem is currently being undertaken by the Department of the Environment's Bureau of Arid Lands Ecology in the Turan Protected Area of eastern Iran. The solution to another environmental problem, dune stabilisation, is also the subject of an experimental approach. Some 5 million hectares of sand dunes, mainly in south-eastern Iran, provide a very unstable substrate which results in low plant cover and productivity. Since 1968, Esso Research Ltd has been spraying dunes with oil to provide a temporary stabilization of surface sand whilst plant life is becoming established. Oil is deposited at a density of about 4 tons per hectare, and the area is then planted with fast-growing shrubs, some of which may attain a height of 2 metres within 2 years and seem unaffected by the oil deposits around their roots. Although the initial effect is unsightly, the final stabilisation is said to be more effective than that achieved by the old system of using brushwood palisades and, in a country where oil is cheap, the method may provide a fast and efficient method of dune reclamation, whether for human or wildlife exploitation.

Plans are meanwhile being put into operation to develop an environmental park, called "Pardisan", within the city of Tehran. A 300 ha site will eventually contain museums, planetarium, aquarium, laboratory complex and a series of reconstructions of various habitats, including fauna, from Iran and other parts of the world. As with the Environment Department's other conservation activities, emphasis is to be placed upon the role of man in the ecosystems portrayed, so the zoological/botanical garden is to be combined with a kind of folk museum in which whole villages will be constructed typical of such areas as the Persian Gulf and Baluchistan. This grand conception, the brainchild of Firouz, has much about it which is attractive, but its practical feasibility remains to be demonstrated.

The one pervasive feature of the Iranian approach to wildlife and habitat conservation is its insistence upon the inclusion of man as a component of all its ecosystems. This country, with about 10,000 years of agricultural history, bears everywhere the mark of man's activities. There are regions which almost certainly could be made more productive if man were to reduce his demand on the environment, but no realistic conservation policy can ignore or totally exclude his presence. It is to be hoped that such enlightened attitudes will penetrate to all levels of Iranian society and will result in the achievement of a balance from which all organisms, including man, will ultimately benefit. ☐

Date palms on northern edge of Great Kavir

California's nuclear war

Californians will shortly give their view on nuclear power. Colin Norman reports on a critical referendum

WHEN California voters work their way through their cluttered ballot papers in the June 8 primary election, they will decide more than the political fate of a handful of Presidential aspirants. They will also determine the future for nuclear power in California and, perhaps, in a number of other states as well.

A proposition, placed on the ballot by petition last year, would set such stringent controls on the construction and operation of nuclear power plants in California that, if passed, it could bring the nuclear industry to a grinding halt there. The outcome of the vote, the first ever plebiscite on nuclear power, is considered so important to the nuclear industry that, together with various supporting groups, it is pouring millions of dollars into a massive campaign to defeat the proposition. California's nuclear war, in fact, is even overshadowing the crucial battle between would-be Presidents for the state's delegates.

The vote will have repercussions extending well beyond California's borders, which is one reason why the matter has assumed such importance. For one thing, voters in several other states will be faced with the same choice in the November Presidential election. Similar propositions have already qualified for the ballots in Oregon and Colorado, they are also considered likely to qualify in North Dakota, Wyoming and Washington, and anti-nuclear groups are hoping to force nuclear referenda to be held in a number of other states next year. The theory is that a victory in California would give a huge boost to the anti-nuclear movement in those states. But, equally important, if the California proposition passes, it could undermine political support for nuclear power in Washington and in state legislatures around the country.

In fact, the proposition's supporters can already claim a victory of sorts. Three bills, considered milder versions of the proposition, are sweeping through the California legislature and are expected to be approved before the primary election. The bills were undoubtedly spawned by the huge publicity given to the campaign, and they would probably never have stood a chance if the proposition had not gathered so much momentum.

Even at this late stage, the outcome of next Tuesday's vote is in some doubt. Although public opinion polls have consistently given opponents of the proposition the edge, the latest poll, conducted in mid-May, indicated that 29% of the voters were still undecided. Of the others, the poll found 30% in favour, and 51% opposed. Those figures are one reason why both sides are conducting a fierce campaign in the final days before the June 8 showdown.

The proposition, and the campaign which is now swirling noisily around it, is an interesting exercise in participatory democracy, and an object lesson in the wild ways of California politics. It will be Number 15 on the ballot papers, buried among questions on issues ranging from bank taxes to legalised bingo.

Formally known as the California Nuclear Safeguards Initiative, Proposition 15 would set the following conditions on the construction and operation of nuclear power plants in California:

- In the event of a nuclear accident, there must be no limit to the total damages which could be claimed by victims. At present, a federal law, the Price-Anderson Act, limits the liability of the nuclear industry to a total of $560 million per accident.
- Within five years of passage of the proposition, the state legislature must have determined, by a two-thirds vote, that nuclear reactor safety systems will function correctly. Such a determination would be made on the basis of tests of the systems conducted "in actual operation".
- Similarly, the state legislature must have determined, by a two-thirds vote, that radioactive wastes can be stored without adversely affecting the health of Californians of their environment.

Unless those conditions are met, no new plants could be licenced and existing plants would be able to operate initially at only 60% of their rated capacity. Their output would then have to be decreased by 10% a year until they are phased out in the mid–1980s.

The proposition qualified for the ballot early last year when its supporters gathered together the necessary 312,000 signatures. The early sponsors of the proposition were a San Francisco-based group called Californians for Nuclear Safeguards and a Los Angeles organisation known as Peoples' Lobby, but much of the original impetus came from Ralph Nader's organisation. An important addition to the pro-initiative forces was the Creative Initiative Foundation, a religious-philosophical group whose lobbying arm, known as Project Survival, joined the quest for signatures to qualify the proposition early in 1975. Three nuclear engineers who quit their jobs at the General Electric Corporation last January to campaign for the proposition were all members of the Creative Initiative Foundation.

Opposition to the proposition is spearheaded chiefly by a committee of representatives from the nuclear industry, businessmen and labour leaders, called the California Council for Environmental and Economic Balance. Headed by Edmund G. Brown Sr, former Governor of California and father of the present incumbent, the committee is well-financed and has hired a firm of campaign consultants to manage its effort.

Because there has been some confusion among Californians stemming from the fact that a 'no' vote on the proposition means a 'yes' for nuclear power, the committees recently changed their names. Supporters of the proposition now campaign under the banner of the "Yes on 15 Committee", while their opponents are headed by the "No on 15 Committee".

The rules, and perhaps the outcome, of the campaign were abruptly changed last January in favour of the opponents of Proposition 15. In the 1974 elections, California voters approved a proposition, again placed on the ballot by the Peoples' Lobby, which would set strict limits on the amounts of money spent by candidates in state-wide elections. But in January, the California Supreme Court ruled that the limits do not apply to campaigns for referenda, and money then began pouring into the coffers of the opposition groups, much of it coming from out-of-state utility and energy companies. By the time the vote is taken the various No on 15 groups will have spent about $7 million, according to some estimates. By contrast, supporters of the proposition will have amassed only about $800,000. Joyce Koupal, who with her late husband Ed Koupal formed the basis for People's Lobby, claimed last week that the opponents are "buying the election".

In the last week or so of the campaign, the No on 15 Committee will sponsor a barrage of television and radio commercials, pushing the argument that the elimination of nuclear power in California will cause unemployment to rise and living standards to fall. And the Yes on 15 Committee will have Ralph Nader stumping the state, arguing that energy can be conserved and alternative sources tapped, in such a manner that living standards will not suffer. Nobel laureates have already been ranged on both sides of the issue, and arguments have been traded back and forth about nuclear safety, safeguards and so on. The California campaign, in short, closely reflects the debate about the merits and hazards of nuclear power which has been raging nationally for the past few years.

But there is one additional issue in the campaign—the problem of operating nuclear reactors in a state where the seaboard is criss-crossed by geological faults, and the interior is almost devoid of cooling water. Because of those factors, experience with nuclear power in California has so far been vexing for the nuclear industry.

There are now three plants in operation, four under contruction, and some 20 more at various stages of planning. Of the operating reactors, one small unit located north of San Francisco is now being reviewed by the Nuclear Regulatory Commission because an active fault has ben discovered close by, while another has been plagued with nagging technical problems ever since it began operating early last year. A storm of controversy has recently arisen over two reactors under construction in Diablo Canyon, midway between San Francicso and Los Angeles, because an offshore fault has been discovered some 3 miles from the site. And it can safely be assumed that similar site problems will arise for future reactors.

Supporters of Proposition 15 thus have a plump target to shoot at, but the opponents are also not short of big guns. One of their chief arguments is that California already imports some 22% of its energy from the other states, and that without nuclear power its dependence on other regions will increase greatly. A substantial source of imported energy at present is a series of dams on the Colorado River, and a number of coal-fired power stations in Utah. But the Colorado is already dammed so heavily that it no longer reaches the sea (it disappears into the sand in Mexico), and Utah residents are growing reluctant to put up with air pollution in order to supply Los Angeles with power. A few weeks ago, for example, the Southern California Edison Company cancelled plans to build a massive coal-fired plant in Utah because of environmentalist opposition. Opponents thus argue that without painful conservation measures and greater reliance on imported oil, California would not be able to meet its energy needs, and it will suffer economically as a result.

One factor clouding the picture in the late stages of the campaign concerns the effect that passage of the three nuclear energy bills by the state legislature will have on the prospects for Proposition 15. The bills, which deal with nuclear safety, safeguards and waste storage (but not with liability limits) would require the recently created California Energy Resources, Conservation and Development Commission to determine whether the federal government has dealt satisfactorily with nuclear power problems, and the commission's findings would have to be approved by a simple majority of the legislature. Unless such approval is given, no new reactors could be built, but the legislation would leave untouched the three existing plants and the four under construction.

Another uncertain factor is whether or not Proposition 15 would survive a court challenge. If the proposition is passed, the nuclear industry would be certain to challenge its constitionality, arguing that it would usurp the federal government's power to set regulations for nuclear power. Legal experts are divided on the likely outcome of such a challenge, but two Stanford University law professors who have studied the matter conclude that most of Proposition 15's provisions would just survive a court test.

If, as now seems likely, Proposition 15 will be defeated by a small margin, it is likely that both sides would claim a victory. In a speech delivered on May 4 at the Forum Atomique Européen in Madrid, Carl Walske, president of the Atomic Industrial Forum, predicted victory for the opponents of Proposition 15, and claimed that such a result would sound the death knell of the anti-nuclear movement. The campaign, he said, "may well be their last big drive".

But if supporters of Proposition 15 come even close to victory, they will claim success, arguing that they were defeated by the industry's money and that the extent of the nuclear opposition is such that there is no mandate for a vast expansion of nuclear power in California. As a spokesman for the Yes on 15 Committee put it last week, the industry knows that it must defeat the proposition heavily, because anything short of a landslide defeat would be called a victory by the anti-nuclear groups. □

Earthquake money

A large helping of money for seismology, together with the establishment of a top-priority programme to reduce the destructiveness of future earthquakes, is promised in a bill approved without debate last week by the US Senate. Proposed by Senator Alan Cranston, the senior Senator from California, the bill has been hovering in the congressional wings for nearly four years; it was rushed to centre stage and swiftly passed last month in the wake of the destructive earthquakes in Italy and the Soviet Union.

If it survives the rest of the congressional process intact, the bill would authorise expenditures of some $150 million over the next three years on a massive programme of research on earthquake prediction, efforts to improve the resistance of buildings to major shocks, studies of the social and psychological consequences of earthquakes, and how to issue earthquake warnings without adding to the disaster.

The bill would also establish a top-level committee of 15 people, appointed by the President, to keep tabs on the effort and to advise the President on its progress, and it would require the White House to prepare annual reports evaluating progress in reducing earthquake risks. Those two provisions would give the effort considerable political visibility. The funds would be shared mostly by the US Geological Survey, which would be authorised to spend $75 million in the next three years, and by the National Science Foundation, whose share would amount to some $60 million.

Describing the measure last week, Senator Cranston noted that 39 states are susceptible to major earthquakes. He also pointed out that a major earthquake in a metropolitan area in California would claim thousands of lives and cost billions of dollars in property damage. "We may be courting certain disaster if we fail to take preventive measures now", he said.

The money is, however, far from being safely in the bank for seismic research. To become law, the bill must also be approved by the House of Representatives before the end of this year. Key legislators there have said, however, that they will hold hearings on the measure as swiftly as possible. The Ford administration is on record as opposing the measure, chiefly because it insists that earthquake research is already proceeding with sufficient priority. Most important, even if the bill is passed and signed into law, the money would still have to go through the annual appropriations process before it can be made available. Nevertheless, the electoral appeal of the measure is obvious and the time is probably ripe for the bill to be approved.

USSR

The clamp-down continues

Instances of harassment of Soviet scientists are still coming to light. Vera Rich reports on recent developments

Soviet "refusnik" scientists dismissed from their posts after applying for a visa for Israel have recently been threatened with arrest. An appeal to the world scientific community, signed by Professor Mark Azbel, Viktor Brailovskii and others, states that some 20 scientists called in to regional police headquarters in Moscow were told that if they did not commence working within two weeks they would be charged under the "parasitism" law, which covers such offences as prostitution, drug addiction, and alcoholism.

The illogicality of this demand—it is the authorities who prevent the refusniks finding work—was compounded by the fact that it was also made of the few would-be emigrants, notably Academician Veniamin Levich, who have not yet been dismissed. This suggests that this particular threat is directed at all those scientists who take part in the informal seminars which the refusniks, including Levich, hold to maintain some kind of scientific life and exchange of ideas. For some time, uniformed and plain-clothes police have been making occasional visits to the seminars; these visits have recently been stepped up, and on one occasion a lecturer was interrupted with a demand for identity cards. One seminar, in Kiev, has been closed down by the authorities.

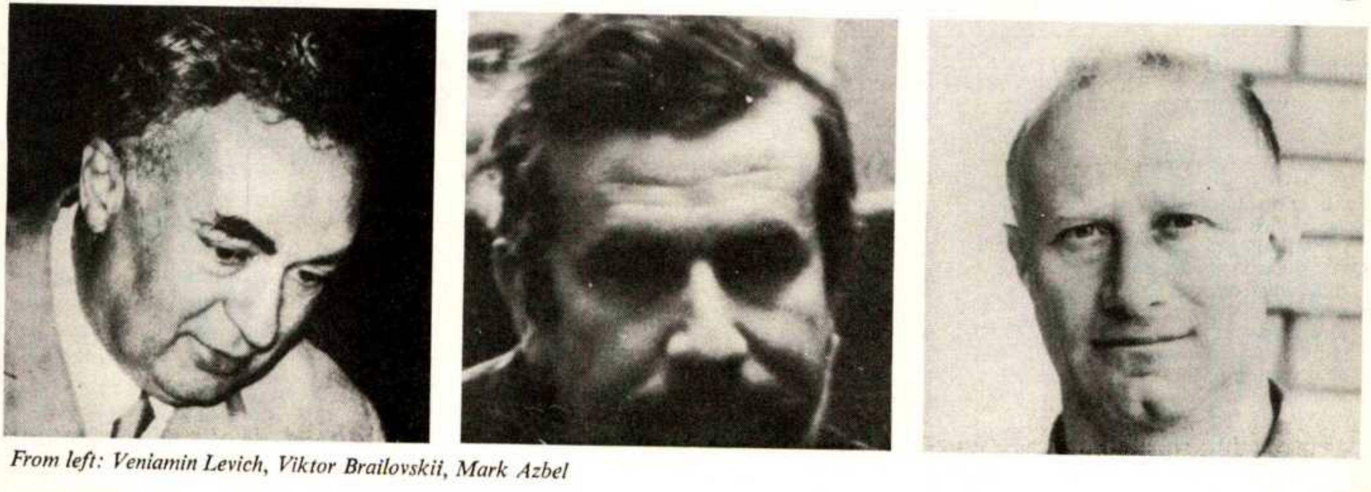
Some idea of the authorities' thinking is also to be found in the recent decision concerning cyberneticists Viktor and Irina Brailovskii. Their original visa applications in 1972 were denied on the grounds that Viktor had had access to classified information. Recently, however, his former superior, Professor Aleksandr Lunts, was allowed to emigrate to Israel; from there he told the Soviet authorities that since his own security status did not preclude emigration, the same should be true *a fortiori* of his former subordinate. Viktor Brailovskii has now received clearance, but Irina has been refused, and has been informed that this is specifically on security grounds. None of her work at the Moscow University Cybernetics Institute was classified, however, as its publication in open journals proves; and since her dismissal in September 1972, there is no obvious way that she could have acquired secret information.

One possible explanation of this anomaly is contained in a recent statement by Al'bert Ivanov on behalf of the Central Committee of the Communist Party of the Soviet Union. Following complaints that the human rights clauses of the Helsinki agreement were not being implemented, Ivanov gave an interview to six prominent activists, including the scientists Mark Azbel, Vladimar Slepak, and Anatolii Sharanskii. Ivanov said that the "terms of 'secrecy' will themselves remain secret. Things which were not secret yesterday might become secret today, and vice versa". Challenged by Azbel that this implied that someone never connected with secret information might be refused a visa in case his work might possibly become secret in the future, Ivanov replied that this was "possible in principle", since each case was decided individually "in the interests of the State"; he further said that there were not, nor would there ever be, any rules on what constitutes "secrecy" or on how long it takes for such "secrecy" to expire.

In their appeal, the scientists stress that pressure from world opinion can effect at least a temporary relaxation of the authorities' attitude: "Our fate and the fates of our families are in your hands, in the hands of our overseas colleagues", they say. Protests abroad, however, can produce retalitory action, as one recent case has shown.

Professor Brian Spalding, Chairman of the Editorial Board of the *International Journal of Heat and Mass Transfer*, had been a welcome guest at the first four All-Union Conferences on Heat and Mass Transfer held in Minsk. Since the last Conference in 1972, however, he has been active in the campaign for Academician Veniamin Levich. In November of last year, Professor Spalding received an invitation to the Fifth Conference held last month, and submitted a paper which was accepted. Academician Levich, however, who developed the now standard electrochemical method of determining heat and mass transfer coefficients, was not invited, and on hearing this Professor Spalding protested to the organising committee of the conference. Academician Levich did, finally, receive an invitation, too late to submit a paper; but Professor Spalding was not given a visa to attend, nor was his paper included in the preprints of the conference.

The Fifth Conference was technically an internal Soviet gathering, to which certain foreign guests were invited, and does not technically come under the declaration of the International Council of Scientific Unions on the free circulation of scientists to international conferences. Nevertheless, since Professor Spalding's paper was accepted, the refusal of his visa is being construed as state intervention in the running of the conference. If the case of Professor Spalding was intended as a warning to the world scientific community, it indicates, as Spalding himself has acknowledged, the constant need for a concerted effort to defend the principles of academic freedom, including freedom of movement and communication for all. □



From left: Veniamin Levich, Viktor Brailovskii, Mark Azbel

correspondence

Selling the Sun

SIR,—It is always welcome when, having written something, others take the time to read and criticise it—positively or otherwise. In your editorial (May 20, page 177) on the solar energy report[1] (to which I was a contributor) you wrote an incisive critique which pointed out some weak points but which I think did not sufficiently stress some of what may be its positive aspects.

We did formulate recommendations on priorities in solar energy research and development—see Chapter 12 of the report, from which the table below is taken.

In drawing up these priorities we studied how the USA, Japan, India, Germany, France, and the EEC had done similar exercises, and then took into account potential use within the UK and the export opportunities—the latter we consider very important in view of the already existing overseas markets and the UK's dependence on export income. Thus it is somewhat unfair to say that we advocated "an advance-on-all-fronts" attitude based only on scientific statements. As to finding figures to back up our claims, we certainly did have problems in obtaining the most up-to-date energy statistics and economic predictions. We did our best in the absence of professional economists and government experts—there are, however, 382 references in the report, and we tried to include the basis for all our facts and assumptions (in marked contrast to many other documents which form the basis for important policy decisions).

Futurology is fraught with dangers. Two government examples in the energy field may suffice. In 1952 it was stated[2] that since selenium solar cells were only 0.53% efficient, "we do not consider that it is necessary to give any special encouragement to the improvement of photovoltaic cells for power production from solar energy". Silicon solar cells are now 16% efficient and we have to import all our requirements for finished polycrystalline and slices for fabrication. In 1965 the CEGB ordered an Advanced Gas-cooled Reactor (Dungeness B) on the assumption that it would be delivering electricity in 1971 costing 0.457 old pence per unit (note the three decimal point accuracy)[3]. In 1973 the cost of electricity from an AGR had risen to 0.57 new pence per unit. Notwithstanding these calculations Dungeness B has yet to deliver any electricity (now costing about 2.0 new pence per unit to the consumer) since it won't be ready until 1978!

Solar energy is not "free" as some may claim. It has problems of collection, storage and transmission—as do most other energy systems to various degrees. The present economic accounting systems discourage investments with high initial costs that provide long term benefits—the case for many important solar energy systems. Detailed systems analyses need to be done to help decide the options for solar energy development—the International Institute for Advanced Systems Analysis in Austria[4] is an excellent example of such work which is immediately applicable to the UK. The construction of demonstration houses, offices, schools and solar equipment, combined with positive incentives to industry and individuals, is the way of the USA[5]. Japan has "Project Sunshine" with defined budgets and objectives out to the year 2000. The UK needs a combination of these approaches if we are to have alternative viable energy options in the next century to be "sold" here and abroad—and not to buy from abroad.

Suggested proportional allocation of research resources in UK for period 1976–1981 with estimates of 1981 possible market split

Application	Suggested proportion of total funds (%)	Estimated market split 1981	
		% in UK	% overseas
Space and water heating	45	80	20
Solar refrigeration and air conditioning	5	5	95
Other thermal applications especially solar pumps, solar engines, solar thermal electric power, etc.	15	0	100
Photovoltaic applications	20	20	80
Bioconversion to fuels and materials* and fermentation of biological wastes	10	40	60
Photochemistry	5	Too early to predict	

*Traditional food production studies excluded

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[1] UK Section Intl. Solar Energy Society, *Solar Energy: a UK assessment*, (21 Albemarle St., London, 1976).

[2] Bullard, E. C., *et al.*, *Research*, **5**, 522–529; 1952.

[3] Patterson, W. C., *Nuclear Power*, 223–224 (Penguin, London, 1976).

[4] Häfele, W., *et al.*, *Second Status Report of the IIASA Project on Energy Systems 1975*, Intl Inst. Advanced Systems Analysis, 2361 Laxenburg, Austria, Report RR-76-1; and *Energy Strategies*, Report RR-76-8; 1976.

[5] Morse, F. H., and Simmons, M. K., *Ann. Rev. Energy*, **1**, 131–158; 1976.

Human anatomy

SIR,—Anthony Harris (Correspondence, May 6, page 10) observed that right limbs tend to exceed the left in various statistics and suggests that this asymmetry is likely to have its origin in the greater use of muscles associated with right limbs. There is evidence, however, that the superiority of the right side is already established in the foetus.

The total ridge count of the finger tips is a quantitative trait which is laid down in foetuses during the third and fourth months of gestational age and thereafter remains unaltered. Although the major component of the ridge count is genetically determined and environmental influences are thought to play only a very minor role, there is a consistent bimanual difference, the mean ridge count on the right hand being higher than that of the left (Holt, S., *The Genetics of Dermal Ridges* (C. C. Thomas, Springfield, 1968)).

An early asymmetry is also seen in the size of the foetal testes and ovaries. The right gonad is larger and contains more cells than the left in foetuses aged eighteen weeks. Although the cause is not known, an explanation in terms of greater use can surely be excluded.

These examples suggest that an asymmetry of development favouring the right side precedes any preference in the use of right sided limbs. It would seem that right-handedness has its origin in developmental anatomy, and that the original asymmetry is subsequently enhanced by differential usage. Cultural factors may increase this difference still further.

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news and views

Not so wealthy stars?

from M. G. Edmunds

PERHAPS the most controversial issue of recent years in the determination of the chemical composition of stars has been the interpretation of the spectra of the so-called "Super-Metal-Rich" (SMR) stars. An interesting analysis of three K giant stars by Ruth Peterson (*Astrophys. J. Suppl.*, **30**, 61; 1976) may help to resolve the disagreement.

The problem may be outlined as follows. The strength of an atomic absorption line in the spectrum of a star is a function of the physical conditions and atomic abundances in the star's atmosphere. Analysis consists of assuming a model of the atmosphere and adjusting the atomic abundances until theoretical line strengths match the observed strengths. It has usually been assumed in abundance analysis that the structure of the atmosphere of a K giant star is uniquely determined by specifying a temperature (on the basis of the distribution of continuum radiation from the star), an acceleration due to gravity and an overall metal abundance—metals meaning all elements heavier than helium. The atmospheric elemental abundances in a K star reflect the composition of the interstellar medium out of which it formed. Most stars have a metal abundance similar to that of the Sun, yet there exist certain stars with absorption lines unusually strong for their assumed atmospheric physical conditions, implying that they have a considerably greater abundance of metals ("SMR") than the majority of stars. Comparison of the luminosities and temperatures of SMR stars with theoretical calculations of stellar evolution has suggested that some of them are considerably older than many stars of lower, solar metal abundance. Thus the SMR stars would not be consistent with the fairly well established near constancy, or slow increase, of the metal abundance of the interstellar medium out of which the stars are born, after a rapid increase in metal abundance during the formation of the Galaxy. So dispute has arisen over whether the anomalous strength of absorption lines in SM stars really does represent an over-abundance of metals.

What Peterson has done is to provide evidence that the line strengths of the SMR stars are consistent with a normal abundance of most metals, but an unusual atmospheric structure. She selected three stars of very similar temperature and surface gravity to minimise the difference of atmospheric physical conditions between the stars. One of them is the SMR giant μ Leo and another the normal giant κ Oph. By obtaining high quality image-tube observations of the spectra she was able to compare the strengths of fairly weak (and consequently difficult to observe) lines between the stars. The use of weak lines is advantageous since it very much reduces the influence on the line strength of uncertain non-thermal velocity fields and atomic collisions. In a K star atmosphere the conditions are such that atoms are either neutral or once ionised. Peterson shows that lines which are from the predominant species in the ionisation equilibrium (a function of ionisation potential of the element) imply that the metal abundance differences between the stars are at most a few percent. Lines arising from the minor species in the ionisation equilibrium are very sensitive to temperature, and it is these lines which differ in strength between the SMR μ Leo and the normal κ Oph. Peterson shows that the difference can be simply accounted for by a lower temperature in the outer layers of μ Leo's atmosphere. She also shows that normal abundances and the alteration of atmospheric structure is consistent with the anomalously strong absorption observed in photometric spectral bands which first suggested the existence of SMR stars.

But why do SMR stars have atmospheric structures different from normal stars? The one element that really does seem to be over-abundant in μ Leo is nitrogen, by a factor of about two.

Peterson speculates that the high nitrogen abundance results in an increased CN molecular abundance in the atmosphere. This molecule could contribute a large number of absorption lines in the near infrared, and by the requirement of energy conservation in the radiation field this will result in a lowering of the temperature in the atmospheric region where the absorption occurs. It remains to be seen if theoretical stellar atmosphere models computed with variable molecular absorption can give quantitatively the required drop in temperature.

There also remains the problem of why the nitrogen abundance is high in the atmospheres of SMR stars. But the excess of this one particular element may prove easier to explain than the previous problem of over-abundance of metals thought to be formed in the extreme conditions of supernova explosion. Nitrogen may be formed in nuclear reactions occurring in the core of moderately massive giants, and it could subsequently be mixed up to the surface. Yet this would imply the reaching of a particular evolutionary stage before a star became SMR. Much work remains to be done to see if all suspect SMR stars are consistent with normal metal abundances except for a nitrogen over-abundance, and to check that such over-abundance can be accounted for by the normal evolution of the stars.

A hundred years ago

WE will leave to other journals the task of criticising the present Exhibition of works of Art at the Royal Academy, and without entering deeply into the question of grouping composition, solidity of painting, *chiaroscuro*, perspective, *morbidezza* of flesh treatment, or aerial effect, we will confine ourselves to a few remarks in a less ambitious key, on those pictures which portray animal life. Of this class there are several important examples devoted entirely to the representation of wild or domesticated animals, with others in which the lower forms of creation play but a slightly inferior part; and in these days when the public taste claims a far more conscientious treatment of the subject than in former times, we may be allowed, without being taxed with unfair criticism to examine how far the respective artists have succeeded in fidelity of execution from *Nature*, **14**, June 1, 105; 1876.

Membranes at EMBO

from M. S. Bretscher

The 2nd EMBO Symposium was held at Hirschhorn-Heidelberg on May 4–8, 1976, and entitled The Structure and Function of Biological Membranes.

THAT the second EMBO Symposium was devoted to "The Structure and Function of Membranes" is a measure of the extraordinary advance made in that area over the past few years. Roughly the first half of the meeting was devoted to transmembrane proteins. These can be divided into two groups: those which extend across the bilayer in a simple manner, such as with a single α-helix—the "fibrous" proteins, and those which have an extensive mass in the plane of the membrane—the "globular" proteins (M. S. Bretscher, MRC Laboratory of Molecular Biology, Cambridge). Examples of the fibrous class include the erythrocyte MN glycoprotein and the spike proteins of Semliki Forest Virus. In the latter case, freeze fracture electron microscopy shows that there are no visible intermembrane particles in the fracture plane, (K. Simons, European Molecular Biological Laboratory, Heidelberg), which fits well with the view that these fibrous proteins have little mass in the plane of the membrane. All the other transmembrane proteins discussed were globular and in freeze fracture are visible as particles.

Had he been present, Walther Stoeckenius would have been pleased to see the progress made on two ordered membrane proteins which he first isolated—the purple protein (bacteriorhodopsin) and gap junctions. The purple protein is induced in a halophilic bacterium when growth conditions become unfavourable. Its assembly can be studied by addition of nicotine to the growth medium which inhibits the synthesis of the cofactor, retinal. Bacterio-opsin is formed which, on subsequent addition of retinal to the culture generates bacteriorhodopsin. In these conditions the bacteria have no purple membranes, however, just a brown membrane in which the bacteriorhodopsin probably exists as single molecules. Formation of the purple membrane seems to be more than just crystallisation of individual molecules of bacteriorhodopsin in the plane of the membrane, as the process requires energy (D. Oesterhelt, University of Wurzburg). The structure of the purple protein, as deduced largely from low dose electron microscope image reconstruction, shows that little of the globular protein extends outside the lipid bilayer. About 70% of the protein can be accounted for by four α-helices which are perpendicular, and three which are tilted, to the plane of the membrane, (R. Henderson, MRC, Cambridge).) So far, X-ray studies of ordered arrays of gap junctions have been hampered by low short-range order. Nevertheless, the electron density profile suggests a rather even concentration of protein across the two bilayers constituting the junction, with about half the intercellular space filled in by protein A tentative model for this array exists in which each pore is composed of twelve subunits: six in each bilayer (D. Caspar, Brandeis University). These two proteins, then, have taken on a defined shape: they no longer are drawn as wursts (objects with which the meeting became too well-acquainted at meals). Chemical characterisation of the gap junction protein is complicated by proteases, so that the subunit molecular weight of its constituent protein(s) is not yet known (N. Gilula, Rockefeller University).

X-ray or neutron diffraction studies of membranes can be aided by having an oriented specimen. Surprisingly, this can be achieved by applying a magnetic field: some membranes, such as erythrocyte ghosts, orientate parallel to the field, others (purple membranes and rod outer segment disks) perpendicular to it. This may be useful, not only for orientating membranes, but for what it tells us about the overall polarity of α-helices in the membrane. Using this ordering technique, only slight changes occur in the X-ray diffraction pattern of rhodopsin in disk membranes on illumination—changes which are too small to be compatible with the model proposed by Blasie in which rhodopsin sinks a long way into the membrane on bleaching (M. Chabre, University of Grenoble).

A major function of specialised membranes is in energy production—pumping protons and using this gradient to make ATP, as proposed by Peter Mitchell. A beautiful system for studying the relation of proton gradients to electron transport and ATP synthesis is closed vesicles of higher plant chloroplasts, in which a gradient of 4 *p*H units can be generated by light. This *p*H gradient behaves as would be expected if it were an energy-storing device between electron transport and ATP formation. In addition, the pathway is reversible—artificially produced proton gradients drive reverse electron flow, which in turn induces light emission (M. Avron, Weizmann Institute).

The ion channel associated with acetylcholine receptors of denervated frog muscle can be studied with electrodes with exquisite sensitivity by limiting the surface area studied to a patch a few microns in diameter. The opening and closing of individual ion channels can be monitored and their properties, such as mean open time and mean conductance, can be determined. These results agree well with, and thus confirm, previous conclusions on the nature of ion channels obtained from membrane "noise analysis", and show that the current produced by a single channel has a square wave form (B. Sakmann, Max-Planck Institute, Gottingen).

The functional organisation of intracellular organelles is one of the most challenging areas of cell biology. A powerful approach to understanding the biochemical anatomy of higher cells is immunofluorescence microscopy, which has been used to special advantage to study the microfilament system in fibroblasts. This reveals that stress fibres contain, besides actin, myosin, tropomyosin and α-actinin. How the assembly of these structures is controlled, however, or why viral transformation (in SV3T3 cells) should lead to a gross diminution in the amount of fibres seen, is unknown. In addition, this technique has been used successfully to look at the disposition of microtubules and their polymerisation

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Fv-1 function

IN the report of April 22, 1976 on the Armand Hammer Symposium at the Salk Institute (*News and Views*, **260**, 669; 1976), it was indicated that our data on the function of the Fv-1 locus of the mouse showed that its product restricts growth of murine leukaemia viruses after integration of the provirus. At the time of the meeting, we had preliminary evidence in that direction which was discussed. More complete investigation, however, has shown that integration is prevented by the Fv-1 gene product although synthesis of proviral DNA is not prevented. Reports of our results and similar results of Sveda and Soeiro are in press in the *Proceedings of the National Academy of Sciences U.S.A.*

DAVID BALTIMORE
PAUL JOLICOEUR

in vivo after disassembly induced by cold or by colcemide. In the majority of cases, individual tubules are seen to grow in a unidirectional manner from the cytocentre of the cell towards the plasma membrane (K. Weber, Max Planck Institute, Gottingen).

Do any of these filamentous molecules interact with membranes? Possibly actin does with chromaffin granules, because when actin is polymerised at 30 °C in the presence of granules, the majority of actin fibres associated with granules show a specific polarity with respect to the granule. Staining with muscle heavy meromyosin shows the arrowheads on most fibres pointing away from the granule (B. Jockusch, Biozentrum, Basel). A different fascinating system in which actin is closely associated with a plasma membrane is found in starfish sperm. On reaching the egg, a 90 μm spike pops out of the sperm head, taking about 30 s to assemble. Artificial triggering, induced by ammonia, allows these spikes to be purified; they consist of an actin bundle surrounded by a limiting membrane. Before erection, this "profilactin" exists in a cup above the sperm nucleus. On isolation, the cup contains just three proteins—actin, and two proteins with molecular weights similar to human erythrocyte spectrin (250,000 and 230,000 daltons). How the spike is assembled and where all the surrounding membrane comes from, is unresolved (L. Tilney, Paris).

The interiors of higher cells abound with membranous structures. The only such component isolated in a pure state (apart from specialised secretory vesicles) is the coated vesicle. By a standard purification procedure, coated vesicles have been obtained from brain, adrenal medulla, parotid glands—all secreting cells—and from a lymphoma cell line, EL4, which is a non-secretor. The major protein in every case is clathrin (180,000 daltons) which seems, from one-dimensional fingerprints, to be highly conserved in sequence. This protein gives rise to polymorphic structures enclosing a vesicle: the outer diameter varies from about 500 Å to 1,200 Å. By tilting stained specimens in the electron microscope, the structures of some of the smaller coats have been deduced—they are made of twelve pentagons and a specific number of hexagons. The function of coated vesicles probably varies between cell types, but is believed to involve membrane transfer between organelles (B. Pearse, MRC, Cambridge).

Activation of cellular processes often results from an extracellular signal. In a simple activation, the time elapsed between signal and effect should be small and this is a virtue of the mast cell: when triggered by antigen, histamine, which is prepackaged in vesicles, is released within seconds. Triggering requires that the antigen crosslink IgE receptors, but ferritin labelling and electron microscopy indicate that no extensive aggregation of receptors occurs in this brief time. Both energy and extracellular calcium ions are also required, and the latter must be present at the moment of antigen addition: later addition of calcium is ineffective. Calcium ions presumably enter the cell as a calcium ionophore alone will trigger mast cells. After triggering, exocytosis of histamine occurs. Ferritin labelling shows that, where the plasma and vesicle membranes become apposed, concanavalin A binding sites are excluded, as are particles seen in the freeze-fracture plane; however, the glycolipid GM1, which can be tagged with ferritin-conjugated cholera toxin, is not excluded from this area. Moments after exocytosis, the site of membrane fusion is confusion—lots of multivesicular blebs (M. Raff, University College London).

How do cells move? Cell locomotion and capping in lymphocytes are presumably related phenomena. If there exists an oriented lipid flow (3 μm min^{-1}) in the plasma membranes of motile cells, a membrane protein complex with a low diffusion coefficient would be capped and, if the complex anchored to a substratum, the cell would move forwards. If the lipid flow were much faster (15 μm min^{-1}), all proteins would cap, which might be unhealthy, whereas if the flow were rather low (0.5 μm min^{-1}), capping of surface antigens would not occur in lymphocytes. In other words, the rate of particle movement on a fibroblast (3 μm min^{-1}) is just right for the lipid flow hypothesis (Bretscher).

It seems to be that the overall problem of the structure of membranes is solved in outline, and specific aspects are well on the way to being solved in detail. But how membranes of a cell are inter-related, how they are organised by cytoplasmic components—that is where future interest lies. □

Blue light for aluminium nitride

from Andrew Holmes-Siedle

LIGHT-emitting diodes composed of electroluminescent materials are used mainly in numerical displays such as mileage indicators in cars, voltmeters and other similar instruments. Considerable interest was aroused in the years 1972–4 when blue and ultraviolet emission from gallium nitride electroluminescent devices was announced (see for example, J. I. Pankove, *J. Luminescence*, **1**, 114–126; 1973; *News and Views*, **252**, 443; 1975). It was a considerable feat of materials synthesis and device design by Pankove's group to make a structure which emitted fairly efficiently in the yellow region at a peak wavelength of 590 nm (corresponding to a photon energy of 2.1 eV) and also emitted some blue light and an ultraviolet line (378 nm, corresponding to 3.28 eV). This was a record low wavelength (that is, record high photon energy) for junction electroluminescence in the III-V compounds, some other members of which are now commercially established as light emitters at lower photon energies (GaAs, GaP, GaAl and their alloys). From the lack of commercial interest in gallium nitride devices since then, however, it seems that no-one particularly needs blue or ultraviolet-emitting diodes at the moment. Perhaps display designers are happy with the green-emitting GaAsP alloys which the human eye responds to most efficiently and the red LEDS, made from GaAlAs, which are perhaps the most arresting. It is likely, however, that a use for blue emitters will emerge, especially in the field of data recording, where the extra photon energy could be needed to induce rapid photochemical effects. Thus, it is not surprising that IBM have attacked a new and formidable peak in the luminescent Himalayas of the III-V compounds, namely aluminium nitride (AlN). This compound is difficult to prepare in suitable form; it is refractory (melting point >2,000 °C) and is normally a good insulator, the resistivity of synthetic single crystals frequently being above 10^{10} ohm-cm. Since the principle of generating junction electroluminescence is to put a crystalline p-n structure under forward bias and pass several milliamps of current, these adverse electrical properties (which are quite consistent with the optical data indicating a forbidden gap of 6.2 eV) have to be somehow circumvented.

R. F. Rutz, reporting in *Applied Physics Letters*, **28** (7), 379; 1975), describes an empirical solution, interesting because it is, perhaps, the result of

Comparison of forbidden gaps, E_g, in III-V compounds

			N	P	As	Sb
Principal quantum number of valence electron			2	3	4	5
		Atomic number	7	15	33	51
B	2	5	5.5 eV	2.0 eV	1.45 eV	—
Al	3	13	6.2 eV	2.42 eV	2.17 eV	1.63 eV
Ga	4	31	3.5 eV	2.24 eV	1.43 eV	0.67 eV
In	5	49	< 1.9 eV	1.29 eV	0.33 eV	0.16 eV

As the energy gap becomes higher the emission moves further towards the ultraviolet.

some useful point-defect structure formed during an unusual type of crystal growth. A very thin crystalline film of AlN was first grown epitactically on a plate of single-crystal tungsten or sapphire by RF sputtering (this would, presumably have to be done very slowly and the crystal would have to be heated). This 'seed' film was then placed very close to a polycrystalline AlN disk in a reactive gas atmosphere and heated to about 1,850 °C, with the seed slightly cooler than the source. A single-crystal film of about $10\ \mu m$ could then be grown and such films had an unusual colour, which suggested a high concentration of nitrogen vacancies. As a result of these vacancies, some of the films had quite a low resistivity (about 10^3 ohm-cm) and they appeared to possess n-type conduction. In this, Rutz's special growth techniques appear to have the advantage over that used earlier by RCA workers (Duffy *et al.*, *J. Electron, Mater.*, **2**, 359; 1973), who grew AlN films on sapphire but report no electrical measurements. The first electrical stimulation experiments by Rutz produced easily visible emission of blue light under the negative electrode, probably as a result of impact ionisation, with a peak in the ultra violet at 3.443 eV (360 nm) and quite strong emission up to 5.76 eV (215 nm), total output for one diode being in the region of a microwatt. The latter emission cutoff value handsomely exceeds the short-wavelength emission record set by Pankove using gallium nitride but quantum efficiency is low as yet, being in the 10^{-6} range.

The practical implications for data recording or display cannot really be assessed yet. The material is obviously more difficult to handle than the low-melting semiconductors, but, on the other hand, aluminium is much more abundant than gallium and the high photon energy opens the attractive prospect that very many phosphors can be excited by these emissions, thereby yielding any colour of display that we wish (this is, after all the principle used both in colour TV and fluorescent lamps) The felicitous use of vacancies to achieve a practically useful effect is also intriguing and will perhaps spur us to look further at the electrical stimulation of some other materials traditionally regarded as incorrigibly 'insulating' such as silica, alumina and—perhaps even the Everest of the III-V compounds—boron nitride. ☐

Ring chromosomes and DNA structure

from Martin Bobrow

THE development of relatively simple techniques for differentially staining sister chromatids in mammalian cells is an important addition to the armamentarium of the cytogeneticist. Latt first discovered (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 3395; 1973) that BUdR-substituted chromatin is less intensely stained by the fluorescent dye Hoechst 33258 than are normal thymidine-containing regions. This can be exploited by allowing cells to replicate twice in the presence of BUdR; at the second metaphase, one chromatid will be fully substituted with BUdR, while the DNA of the other chromatid will have the original thymidine-containing strand and a newly synthesised BUdR-containing complementary strand. Differential staining of the two chromatids can be achieved using Latt's original fluorescent technique; by staining with Hoechst 33258 followed by Giemsa (Perry and Wolff, *Nature*, **251**, 156; 1974); with Giemsa alone after heat treatment (Korenberg and Freedlender, *Chromosoma*, **48**, 355; 1974), or by acridine orange staining (Kato, *Nature*, **249**, 552; 1974).

These techniques elegantly demonstrate the presence of sister chromatid exchanges. Such exchanges are a regular feature of normal cells Their frequency is greatly increased in an inherited disease (Bloom's syndrome—Chaganti *et al.*, *Proc. natn. Acad. Sci. U S.A.*, **71**, 4508; 1974), and they are a sensitive indicator of certain classes of chromosome-damaging agents (see Savage, *News and Views*, **258**, 103; 1975). It is generally presumed that exchanges between sister chromatids reflect in some way a mechanism of DNA repair, and it is tempting (although still very speculative to draw analogies with post-replication or recombinational repair mechanisms in bacteria.

An unusual and imaginative use of sister-chromatid staining techniques has been reported recently by Wolff, Lindsley and Peacock (*Proc. natn. Acad. Sci. U.S.A.*, **73**, 877; 1976) to lend support to a controversial notion that there may be switches in polarity within DNA chains. Ring chromosomes which undergo a sister-chromatid exchange become a single continuous chromatin strand, since the exchange links each chromatid to its sister. At subsequent metaphases, such a chromosome appears as a dicentric, with its centromeres symmetrically placed, and a circumferential length twice that of the original ring chromosome. The principles of ring chromosome behaviour are those of the Mobius strip (well described by Gardiner, *Scientific American*, **219**, 112; 1968).

Wolff and his colleagues studied ring chromosomes induced by irradiating Chinese hamster cells in tissue culture. Conditions of culture were such that sister chromatid exchanges involved in the formation of the dicentric rings could be directly demonstrated. Cell division after irradiation was inhibited by colcemide, to prevent the dicentric chromosomes being disrupted on the mitotic spindle. The majority of the dicentric ring chromosomes with symmetrically placed centromeres did show the presence of a sister chromatid exchange as expected. A substantial minority, however, showed no such exchange present. After an exhaustive consideration of possible errors in scoring, and of the various mechanisms of ring chromosome formation, the authors conclude that such a ring could only be formed if the original chromosomal DNA (before ring induction) had contained a switch in polarity. The effect of a switch in polarity would be that when the broken ends of the irradiated chromosome rejoin to form a ring, the normal constraints of DNA structure would ensure that each chromatid end became continuous, not with its own tail, but with that of its sister. So in effect sister chromatid exchange and ring formation would be accomplished simultaneously, at the time of ring induction.

The authors believe that such reversals of polarity are an integral part of the structure of the chromosome, and estimate their frequency at one in 10^9 phosphodiester bonds. They could conveniently occur at positions of protein links in a DNA chain, but the existence of such links is in considerable doubt The evidence today points towards each chromatid containing a single continuous DNA chain, in which case a reversal of polarity must imply the existence of 3′-3′ and 5′-5′ bonds.

It is obviously hazardous, in our present state of ignorance, to attempt to explain cytological phenomena at a molecular level. The explanation proposed does, however, fit the observation and no alternative explanation springs readily to mind. Whether the particular hypothesis is or is not eventually proven correct, the work of Wolff, Lindsley and Peacock is a fine example of how much of interest can be gleaned from intelligent microscopic observation of chromosome behaviour.

T4 chromosome

from a Correspondent

THE complex chromosomes of higher organisms are tightly organised, their DNA being coiled into standard "beads" which contain 200 base pairs associated with specific histone proteins. Recently the DNA of bacteria has also been shown to have a similar structure, although it is stabilised by a different kind of protein. Do bacteriophages, which are probably the simplest type of living organism, also replicate their DNA in a highly structured manner? In phage T4 it has been known for some time that intracellular phage DNA must be organised in some way, as the rate at which it sediments during centrifugation (about 700S) would reflect DNA concatemers with lengths of nearly 1,000 T4 genomes if attached end-to-end; but in fact the concatemers are at least 10 times smaller than this. The intracellular DNA must therefore be tightly folded and/or attached to some type of non-DNA molecule.

A surprising property of these structured T4 DNA molecules is their stability. Unlike the chromosomes of eukaryotes or bacteria their sedimentation rate is unaffected by treatments with protease, RNase, heat or detergent. One method of affecting this stability has been found however. When bacteria infected with a temperature-sensitive phage mutant in gene 32 (which controls the synthesis of unwinding protein) are grown at low temperature, normal intracellular DNA sedimenting at 700S can be isolated; but within one minute of shifting these infected cells to 42 °C the phage DNA is found to have an appreciably lower sedimentation rate which is approximately that of T4 DNA which is now only one genome in length.

Curtis and Alberts (*J. molec Biol.*, **102,** 793, 1976) have studied the reasons for this dramatic change in sedimentation behaviour in detail. They have shown that along the DNA concatemer which is formed during T4 replication there are sizable single-stranded regions spaced at average intervals of approximately one genome. Normally the product of gene 32 binds tightly to these single-stranded regions and protects them from nuclease action. This protection is rapidly lost if the 32 protein is inactivated, leading to strand scissions and the formation of phage DNA of single genome lengths.

On its own this new information concerning the existence and regular spacing of the single-stranded regions does not explain the higher sedimentation rate of normal intracellular T4 DNA. It does however suggest that single-stranded regions play a key role in holding the T4 DNA in a tight configuration, perhaps by attachment to some non-DNA substance in a way not too unlike the situation obtaining with the chromosomes of higher organisms and bacteria. As Curtis and Alberts point out, some high degree of order in the structure of DNA might be required to guide the simultaneous processes of DNA transcription, replication and recombination—and in the case of phage, of maturation as well.

Facts of colonial life

from a Correspondent

A meeting on the Biology and Systematics of Colonial Organisms was held at the University of Durham, on April 7–8, 1976, organised by the Systematics Association.

MANY organisms live in colonies but the varied nature of coloniality suggests that such associations are influenced by an interplay of factors among which cooperation, competition for space and reproductive and feeding requirements are most significant. At the meeting the phenomenon of coloniality was traced from bacteria, through bryozoans and echinoderms, to sea birds in an attempt to discover what common factors link the phenomena of association, gregariousness and coloniality. Groups of animals of the same species may live in close proximity (gregarious colonies) and so form associations which differ from the integrated growing-together of "conventional" colonial organisms (corporate colonies).

The significance of cooperation was illustrated at microbial level (M. J. Carlile, Imperial College, London) for bacterial colonies. These aggregations are more able to withstand desiccation and show enhanced rates of enzyme production, improving substrate attack and serving as a barier between colonies of different ages and genotypes. Cooperative coloniality occurs also in protozoans where, in more advanced taxa, some parts of colonies are already functionally specialised for gamete and spore production (C R. Curds, British Museum (Natural History)). Proto-cooperation may be significant in gregarious colonies as in the bizarre and extinct, sedentary, rudist bivalves which maintained growth away from their substratum only with the support of contiguous neighbours (P. W. Skelton, University of Liverpool).

Higher levels of cooperation are seen in corporate colonies in which specialised polymorphs, or groups of polymorphs (cormidia) function at several hierarchically cooperative levels This is spectacularly marked in byrozoans where an increasing number of colonial structures have been recognised as modified zooidal features common in colonies with very high levels of integration (J. S. Ryland, University College of Swansea) Colony-wide feeding and maintained current systems may be related to these levels of integration (P. L. Cook, British Museum (Natural History)).

By contrast, the level of cooperative coloniality in sponges is uncertain. Grafting experiments (A. S G. Curtis, University of Glasgow) indicate that clones or clusters of sponges do not exhibit corporate coloniality. Grafts from a single sponge body will be accepted by another only if they are of the same strain. Similar strains will coalesce if they grow adjacent to one another. Although it was argued (W. G. Fry, Luton College of Technology) that a sponge is a colony in which aquiferous units represent individuals it is evidently difficult to define the individual in sponge morphology and the implication of Curtis's experiments were of wide interest to participants at the meeting.

Reproduction as a factor in coloniality is significant in that most corporate colonies develop by asexual budding. The factors controlling the timing of the appearance of new individuals are not known Indeed no obvious relationship to age or maturity for asexual budding can be deduced in, for example, rugose corals. Asexual reproduction in corporate coloniality links closely with the cooperative function but sexual reproduction may itself be a cause of aggregation in some organisms. Barnacle aggregations relate to their internal fertilisation requirements since they have to settle sufficiently close together for this to occur (D. J. Crisp, University College of North Wales). Although external fertilisation occurs in the sessile marine gastropod group of the vermetids proximity seems equally necessary for their survival (R. N. Hughes, University College of North Wales). Reproduction is not, however, so basic a factor as environmental conditions in the spacing of serpulid worms since the same species may occur singly as well as in aggregations (H. A. Ten Hove, State University of Utrecht). This is also shown by clustering of serpulids in Ardbear Lough, County Galway, where they aggregate densely on restricted areas of suitable substrate in otherwise favourable parts of the Lough (D. W. J. Bosence, Goldsmiths' College, London).

The final paper of the meeting (J. C. Coulson and F. Dixon, University of Durham) linked coloniality and sexual reproduction in a study of kittiwake colonies in which the protection and stability afforded by colonial association supports successful and constant breeding pairs in inner areas whereas in outer areas of colonies breeding and adult survival rates are lower and divorce rates higher. Even so the system is not inbreeding because young birds are recruited from other colonies.

The role of suspension feeding in coloniality is important for echinoderms (G. F. Warner, University of Reading) and invites generalisation for other groups also. Suspension feeding allows crowding of aquatic benthonic organisms but, as in the case of starfish, dense aggregates may be a predatory response to food shortage.

Competition for space was mentioned by many contributors but discussed in its own right by J B. C. Jackson (The Johns Hopkins University). In an analysis of the relative success of species on hard substrata of tropical reef environments the particular success of corporate colonial organisms in two aspects essential for establishment was emphasised—effectiveness in competing for space (outward growth) and effectiveness in establishing a particular level in the water column for food supply (upward growth). Thus colonials always prevail over solitaries even though colonials generally settle later. Solitary sedentary species hold their own if they aggregate in the ways described for barnacles, vermetids, rudists and serpulids, or if they grow upward.

For the four main themes of the meeting the starting point is cooperation. In marine organisms this is made possible (necessary for some) by the suspension feeding habit. Sedentary marine organisms must also compete for space. Alternatively competition for space also leads to gregarious coloniality which arises when sexual reproduction rather than cooperation alone may be significant. Such generalisations derive from consideration of the groups referred to at the meeting. These also included coelenterates, ascidians and graptolites but not social insects, fish schooling behaviour or gregarious and social behaviour in mammals. Perhaps cooperation, space competition, feeding and reproduction are also consistent themes which can be carried through to the most complex colonial/social species of all, *Homo sapiens*. ☐

Informatics

from Kevin P. Jones

Informatics 4, organised by the Aslib Co-ordinate Indexing Group, was held on April 5–7, 1976 at the University of Lancaster. The proceedings will be published by Aslib.

THE original Russian definition of informatics, by Mikhailov, Chernyi and Giliarevskii (*Osnovy Informatiki*, Moscow, 1968), that it is the learned discipline which studies the structure and properties (but not the actual content) of scholarly-information activity, its theory, history, methodology and organisation, provided the starting point for several papers. If this definition is taken with R. A. Fairthorne's assertion (*Informatics 3*, Aslib, in the press) that the target for informatics should be the automation of skills based on linguistic experience, together with the fact that the conferences have been deliberately interdisciplinary, then it should be possible to gain some impression of what the *Informatics* conferences have set out to achieve.

The inter-relationships between linguistic structure and meaning have provided a dominant theme through all the conferences. These may be traced in Margaret Masterman's (Cambridge Language Research Unit) contributions, which have developed the hypothesis that as it is now technologically feasible to store what amounts to a 'library' on magnetic disks then it is desirable to develop techniques for extracting information from them while retaining its original meaning. Her theory has explored the automatic partitioning of text by the use of rhetorical figures and the applicability of LIST processing languages, notably LISP 1 5, to semantic structures.

The analysis of structure within large textual units was explored by W. John Hutchins (University of East Anglia). Clearly the elucidation of this structure must form the basis for decisions on what a text is about in both manual and automated systems. Interestingly, this paper, which had been developed from the standpoint of indexing need, shared common ground with Yorick Wilk's (University of Edinburgh) exploration of frame theory from artificial intelligence: both commented on story structures. Wilks was critical of frame structures, particularly those which attempt to map the system's (or robot's) environment as the structures tend to alternate between inadequacy and overcomplexity. It is clear that it is going to be extremely difficult to mirror by machine man's ability to focus on his environment and correlate this with earlier experience.

Two lively interactive systems were described. One was developed at the University of Aston by John Fisher (Herriott Watt University) as part of his development of a *Functional Theory of Language*. Verbs, notably an explicitly performative class, achieve an unusual prominence in Fisher's theory and careful categorisation of these has enabled highly inferential programs to be written which are capable of generating a genuine man–machine dialogue. Robert Oddy's (University of Aston) novel approach to information retrieval bridged the narrowing gap between artificial intelligence and information retrieval. Oddy's program replaces normal Boolean search strategies by an automatic modelling technique which attempts to reflect the enquirer's needs by constructing large semantic networks.

The history of the thesaurus, or conceptual wordbook, was reviewed by Tom McArthur (Longmans), from Bishop Wilkins through Roget to the author's *Lexicon of Contemporary English*. Thesauri have been widely exploited in the design of information retrieval systems to provide semantic frameworks for assisting indexing and searching operations.

Informatics originated in the USSR and it was pleasant that Professor Vladuts was present at the conference, from VINITI—his formal contribution consisted of a survey of current Soviet research within information languages.

Fairthorne, who has been closely associated with Informatics in the United Kingdom, has frequently castigated librarians and information scientists for their disregard for theory. Informatics 4 not only showed that a broad base for theoretical development is fully justified, but that the careful application of theory can lead to great improvements in practice. Peter Noerr (British Library) has applied computers to a bibliographical classification using relational operators, an operation thought to be impossible only a few years ago. ☐

reviews: telecommunications

Trends in optical fibre transmission research

J. E. Midwinter*

Recent advances in the manufacturing technology of low loss optical fibres have made possible serious systems design and testing. But this has highlighted the fact that the mechanisms involved in the propagation of light in multimode fibres were at best, poorly understood. New measurement techniques are now being used to check more sophisticated theoretical analyses of propagation in "imperfect" and cabled fibres and are leading to a deeper understanding of this new transmission medium, its possibilities and its limitations.

THE invention of the laser in 1961 and particularly the GaAs laser in 1962 led research workers in the telecommunications field to examine the possibilities of using light in place of electricity for signal transmission. Free space propagation could be ruled out for civil use because of the relatively frequent occurrence of fog, smoke or rain causing interruptions, and at a fairly early stage, glass fibres were proposed as a suitable medium for guiding the light from source to detector. Stimulated, no doubt, by the observation that the carrier frequency of light is very large (10^{15} Hz), the early system proposals tended to be centred on very wide band transmission, typically 10^8 to 10^{10} Bit s^{-1} combining single mode fibres and lasers to form a sophisticated transmission system. Now, a decade later, real systems are beginning to emerge from the laboratories and surprisingly to the outsider, the intervening research has led to less exotic specifications with most systems designed to operate in the range 8–140 MBit s^{-1} using very overmoded fibre and in many cases with an incoherent light-emitting diode source in place of a laser. But at the same time, this apparent down-rating of the performance has been accompanied by an increasingly more detailed understanding of the transmission medium, its limitations and possibilities.

The shift to lower data rates results from a careful appraisal of the real needs of the telecommunications networks and also from a rather more sober evaluation of the capability of complete systems as compared to the theoretically best performances attainable from individual components. The GaAs laser remains a most attractive source, both on grounds of ease of modulation, through its drive current, and because of its near ideal wavelength coinciding with a minimum in the loss spectrum of the fibre. Interest also centres around the use of light-emitting diodes (LEDs) which currently offer rather longer life expectancy and sufficient performance when set alongside lower bandwidth requirements and more sophisticated fibre designs.

An LED source requires the use of a large core diameter (60 µm) fibre if sufficient power is to be launched, while it is much easier to launch a laser efficiently into a large core fibre than into a single mode (3 µm core diameter) fibre. Finally, the need to join fibres in a field environment adds further to the attraction of using large core diameter fibres for operating systems, although maximum performance will only be obtained using the very low dispersion single mode fibres. At present, however, a great deal of work is being devoted to understanding in depth the multimode fibres, waveguides which allow several thousands of electromagnetic modes to propagate with the energy spread nearly uniformly among most of them. This is to be contrasted to most microwave waveguide systems in which either the waveguide is designed to propagate only a single electromagnetic mode, or the launching and guide design are such as to favour strongly one mode only (as in the TE_{01} millimetre waveguide used for telecommunications). It should also be noted that in optical systems, one is concerned with waveguide propagation over distances of the order of 10 km using a wavelength of 10^{-4} cm; that is, over a distance of the order of 10^{10} wavelengths. Since the equivalent guide length in a centimetre waveguide would be 100,000 km we can expect to find a different emphasis in our study of the optical guides.

The early studies of the very overmoded optical fibre guides used a simple ray analysis to study the details of propagation but more recently more sophisticated approaches have been developed which still avoid the frightening complexity of a full electromagnetic wave analysis but which allow study of details such as the effects of different types of mode coupling and of core-cladding refractive index profiles which do not involve simply a sharp interface but which include diffusion or controlled rounding. Very recently, this analytical advance has been supported by experimental advances which promise to make possible the intimate study of overmoded guides, mode by mode, even when several thousand modes are present.

Before discussing these matters, however, we should note that this interest has only arisen since it has become clear that optical fibres will be available with very low losses and to reasonable tolerances (a few % in most vital parameters). Fibre losses in the range 2–10 dB km^{-1} have been produced by a number of distinct processes. Systems designs begin to be most attractive once fibre losses drop below about 7 dB km^{-1}.

Three fibre manufacturing processes seem to be in contention. The Corning Glass Company have described the "soot" process in which $SiCl_4$ with or without some dopant is reacted with oxygen in a flame to generate a stream of ultrafine and ultrapure silica particles which are deposited on a suitable

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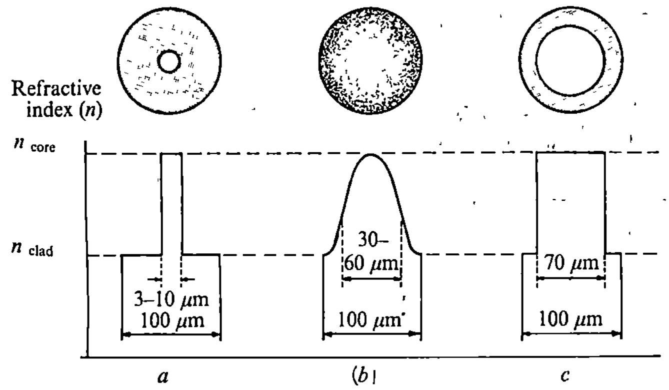
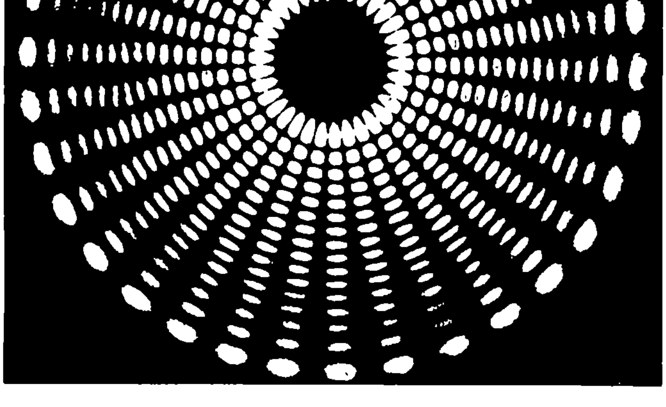


Fig. 1 Cross sections of the major fibre types showing (*a*) small core monomode fibre, (*b*) large core step-index multimode and (*c*) large core graded-index fibre. In all cases the index difference between core and cladding is about 1%.

mandrel. Two thick layers of doped silica are built up and sintered, the mandrel is removed and the resultant mass collapsed to form a preform having an inner core of higher index material surrounded by lower index material with differing dopant concentration. This is then pulled in very controlled conditions to yield fibre. The second process is very similar, except that the gases are reacted in a hot zone within a silica tube, so that layers are built up on the inside surfaces of the tube. The tube is then collapsed to form a preform and pulled to yield fibre. Both processes have the attraction of working directly from the vapour phase to yield very high purity solid material. This is vital because the principal loss mechanisms in glasses in the 800–900 nm wavelength band are absorption by iron, copper, nickel and manganese contamination.

The remaining process relies on glass made from oxide powders, produced in more or less conventional conditions in bulk. It is then converted into fibre using two concentric crucibles both with nozzles in their bases to give two concentric glass streams from which a fibre can be drawn. In this process exceptional care must be taken not to allow any contamination to enter the pure starting powders used, and chemical means must be used to maintain the impurities that are present in the most favourable oxidation state if their absorption loss is not to be unacceptably high. The process does have the attraction that it is immediately usable for continuous production, since glass can be loaded into the crucibles at the same time that fibre is being drawn, whereas preform processes are run in batches and are frequently limited to generating lengths of only a few kilometres of fibre per preform.

Fig. 2 The mode pattern of an $LP_{17,16}$ mode (kindly supplied by W. Stewart of Plessey Co.).



Given low loss fibre, which is essential if a fibre communications system is to compete with existing coaxial cable systems, then the fibre must be engineered into a system in such a way as to allow useful amounts of information to be transmitted over the long distances made possible by the low loss, typically 5–10 km of fibre between source and detector. Thus the dispersion of the fibre becomes significant.

If one focuses attention initially on a step index, multimode fibre as shown in Fig. 1*c* then one can imagine light propagating along the higher refractive index core structure by total internal reflection at the core cladding interface. Since it is a large core diameter fibre, the energy can travel in any one of several modes, zig-zagging at a large angle or a small angle to the axis, or following various helical paths. These modes or paths are of different length and suffer different time delays. Since in practice, most will be excited, very large pulse dispersion occurs, so limiting the available bandwidth. For all modes excited equally, the r.m.s. pulse width after length L is given by

$$\tau = \frac{LN\Delta}{2\sqrt{(3)}c}, \quad \Delta = (n_{\text{core}} - n_{\text{clad}})/n_{\text{core}}, \quad N = \text{group index of core} \tag{1}$$

This amounts to 15 ns km^{-1} for a value of $\Delta = 0.01$ which is typical. Such dispersion over an 8 km link would limit the 3 dB bandwidth to about 4 MHz.

Measurements of long lengths of step-index fibre show larger bandwidths than would be predicted by the above result. This arises from two factors, namely partial excitation of the available mode spectrum and mode coupling. If the source only excites the lower order (low angle) modes of the fibre, then the slowest modes will carry no energy and less pulse dispersion will result. Impressive bandwidths have been reported in such conditions but they are probably not of much more than academic interest since a fibre packaged into cable will in all probability be subject to mode coupling from the impressed lay deformations. This leads to subtle effects.

In the first instance, the launched energy spreads out among the available modes, populating both the slow travelling and fast travelling, so that compared with the earlier example, an increase in pulse dispersion will occur. But if the fibre was initially excited by an incoherent source such as an LED, most or all modes will have been populated from the start, so that the possibility of a low initial dispersion does not arise. Then the immediate effect of mode coupling is to start interchanging energy among modes. In this situation, after a certain distance, it can be said that any particular packet of optical energy will have spent some portion of its time in every mode, so that its mean group velocity starts to approach an average value, which is independent of the mode launched.

To describe theoretically such a state of propagation, it is necessary to make some assumptions about the nature of the mode coupling between the guided modes and the loss mechanisms that will be active within the fibre. Estimates can then be made of the pulse dispersion in such a situation. At this point some experimental guidance is required but before examining the data, we should refine our concepts of modes in the circular dielectric guide.

We have already commented that energy can propagate along zig-zag or helical paths in the fibre, with successive reflections at the core-cladding interface giving the guidance. Interference restricts the angles at which propagation can occur and so defines the "modes". The modes can best be described using the linearly polarised (LP) notation. An LP_{mn} mode is easily recognised as one having n field maxima along the radius vector and $2m$ field maxima around a diameter. The modes are all circularly symmetrical. Figure 2 shows an LP mode field pattern photographed on the end of a 60-μm core diameter fibre, with the core cladding interface corresponding roughly to the outer edge of the field pattern. The integer m is a measure of the degree of helical propagation, the greater m the tighter the helix, whereas the integer n relates to the angle the waves make to the fibre axis in the r–z plane. Any particular LP_{mn} mode in a given fibre can be characterised by a precisely defined value of propagation constant along the fibre axis or z direction, signified by k_z. Modes for which $(2n+m)$ are equal have roughly the same value of k_z, a fact which leads to the frequent use of a reduced mode number $M = (2n+m)$.

A study of the experimental data on mode coupling in fibres leads to the conclusion that the effects observed can best be described by couplings between modes having $\Delta M = \pm 1$ or nearest neighbour modes. Theoretical studies in which the likely mechanisms to induce such coupling have been considered suggest selection rules of the type $(\Delta m = \pm 1, \Delta n = 0)$ or $(\Delta m = \pm 1, \Delta n = \pm 1)$. Evidently the earlier conclusion includes the latter but is rather broader. To produce coupling of the latter type, all that is necessary is a small deviation of the fibre axis from the straight path. The deviation takes the form $\Delta x = d\cos(\Delta k.z)$ where z is the direction of the fibre axis. For strong coupling to occur between modes having values of k_z given by $k_{z,1}$ and $k_{z,2}$ the relationship

$$\Delta k = |k_{z,1} - k_{z,2}|$$

must hold. In addition, the values of m and n for the mode pair must satisfy the selection rules above. Such restrictions sound sweeping, so that one might imagine that such couplings would be unlikely to occur, until it is noted that the values of Δk that are operative are typically 10–100 rad cm^{-1}. Therefore wavelengths in the millimetre to centimetre range are important while d can be less than 10^{-4} cm! Such deviations can easily occur in a fibre packaging process, or even in winding a fibre on a drum so that we find in practice that such mode coupling is often present in fibres.

Evidently, coupling between different mode pairs will require different values of Δk_z. It is found that to couple modes with low values of M, smaller Δk_z values are required than for larger M values. This fact opens some possibilities and brings us to some problems[1]. Figure 3 shows the allowed values of m and n for guidance to occur with a given fibre. Modes exist outside the boundary but they are lossy. Alternatively, one can say that the rays corresponding to the m,n values outside the guided-mode boundary are not totally internally reflected at the interface. If the mode coupling occurs in the fibre because of randomly distributed microbends, as deduced earlier, then we must assume that not only will guided modes be coupled to guided modes, but guided modes will also be coupled to leaking modes, resulting in an increased loss for the fibre. Experimentally this is found to be the case and the observations fit quite well with theoretical predictions.

Mode coupling is expected to reduce the pulse dispersion after an initial fibre length in which the first group velocity averaging takes place. If the additional loss due to the mode coupling is δ dB km^{-1}, then the initial distance for averaging is roughly given by $L_c = 1/\delta$. Up to that point, the pulse dispersion is described by the formula of equation 1. Afterwards, the pulse dispersion is reduced by a factor R where

$$R \simeq (1/\delta L)^{\frac{1}{2}} \tag{2}$$

so that from this point on, the pulse width only increases as $\sqrt{L}$ instead of L. The effect of this can be very significant on long fibre lengths, as is shown in Fig. 4, in which the pulse dispersion is shown for a 1% index difference fibre with varying degrees of random mode coupling introduced. The curves are theoretical, but good general agreement with experiment is found. The almost inevitable presence of a small level of mode coupling in a cabled fibre thus leads to a considerable uncertainty as to how it will perform when in a very long length, such as is expected to be used in operating systems. This inevitably raises questions about the stability of such mode coupling with time and the extent to which it can be exploited to enhance the fibre performance. Answers are still being sought.

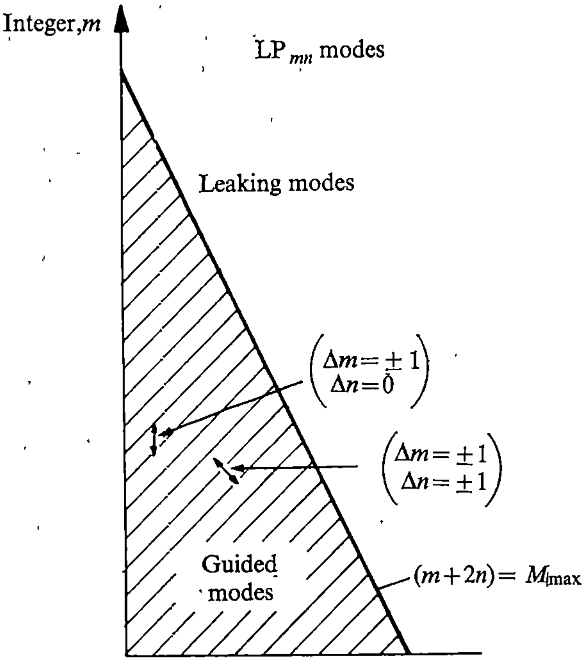
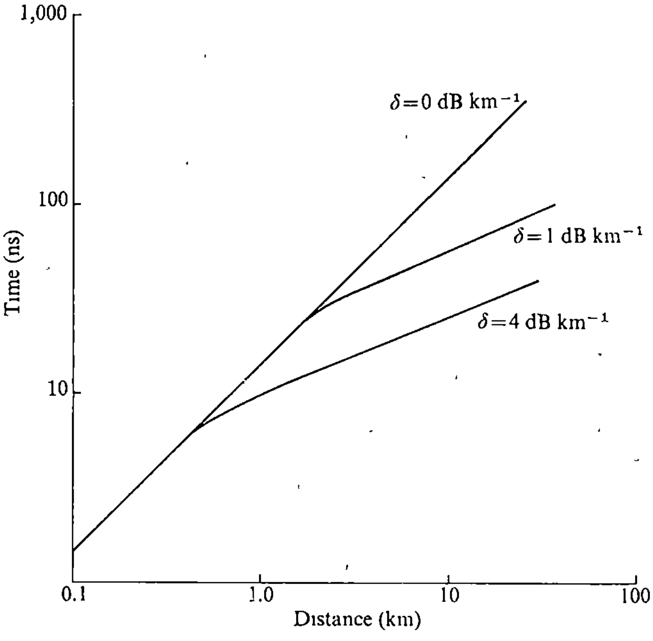

Integer,m
LP_{mn} modes
Leaking modes
$\begin{pmatrix}\Delta m = \pm 1\\ \Delta n = 0\end{pmatrix}$
$\begin{pmatrix}\Delta m = \pm 1\\ \Delta n = \pm 1\end{pmatrix}$
Guided modes
$(m+2n) = M_{max}$
Integer,n

Fig. 3 The allowed values of m and n for the guided modes of a multimode fibre, showing the selection rules for coupling due to fibre microbends.

We alluded to another interesting possibility that arises from this line of thought. It should be possible to introduce selective coupling only between guided modes of a fibre if the deformation spectrum (Δk) can be controlled. It is at least theoretically possible to introduce desirable amounts of mode coupling without loss but with improved bandwidth. To do this, the higher frequencies present in the spatial deformation must be cut off just short of the values at which coupling across the guided mode–leaking mode boundary occurs. Experimentally this has not been done, although experiments have been reported in which chosen pairs of modes have been selectively coupled together by the use of an impressed deformation. It remains to be seen how far the technique can be extended.

Fig. 4 The pulse broadening predicted by equation 1, both with and without small levels of mode coupling present. Calculated $\Delta = 0.01$.



A quite independent approach to achieving increased bandwidths with large core diameter fibres has been to develop fibres in which the core cladding interface is not sharp but is diffused or graded, as illustrated in Fig. 1*b*. The effect of such a profile rounding is readily understood, if we consider the ray paths for the three primary modes of propagation. A central ray travels in the core centre, in high index material by the shortest possible path but relatively slowly. The meridional ray, crossing the central axis, is focused back and forth along a near sinusoidal path, partly in low index and partly in high index material along an intermediate distance path but with a mean velocity given by some average of the indices traversed. Finally, the helical path ray travels the longest physical path, but wholly in low index material and thus at the highest mean velocity. Evidently, the path length divided by the mean velocity is to some extent compensating for each. Mathematical analysis, using the WKB[2,3] method shows that a very high degree of compensation is possible provided that the index profile can be sufficiently well controlled. The form of profile that has received the most attention, termed the "alpha" profile, is as follows

$$n(r) = n_0\,[1-2\Delta\,(r/a)^{\alpha}]^{\frac{1}{2}} \quad a > r > 0 \tag{3}$$

$$n(r) = n_0\,(1-2\Delta)^{\frac{1}{2}} \simeq n_0\,(1-\Delta) \quad r \geqslant a \tag{4}$$

It is an attractive one to study theoretically, not only because analytical results can be obtained for it but also because it encompasses a range of profiles of experimental interest. For example, $\alpha = \infty$ corresponds the step-index fibre discussed earlier, whereas $\alpha = 2$ corresponds to the parabolic dielectric constant profile that was the subject of much earlier study.

The outcome of these studies has been to show that the optimum profile is one that is very close to parabolic ($\alpha = 2$). The pulse dispersion in such a profile with all modes excited is decreased over the step-index profile by a factor of approximately $(4/\Delta)$ so that for a 1% index difference fibre, an approximately 400 times decrease in dispersion is possible in theory. Experimental results approaching this have been reported. An impressively low figure of 0.18 ns km^{-1} has been measured on a 1 km length of fibre, caused by mode dispersion. This represents an improvement factor of some 80 times over the step-index equivalent fibre. To obtain such performance requires, however, exceptional control of the fibre preparation stages and it has yet to be demonstrated that that is possible in large scale production. Against this, it must be said that many systems currently envisaged do not require such low dispersion in a fibre. It follows that fibres with carefully controlled index profile are of great interest at present, with many groups involved in the study of their detailed propagation characteristics, measurement of their vital parameters such as the actual profile obtained, together with intensive studies of manufacturing techniques for such fibres. The gains that can be achieved are illustrated in Fig. 5 which shows the theoretical dispersion curves for fibre with fixed $\Delta = 0.01$ but different values of α. Relatively little profile rounding greatly decreases the mode dispersion.

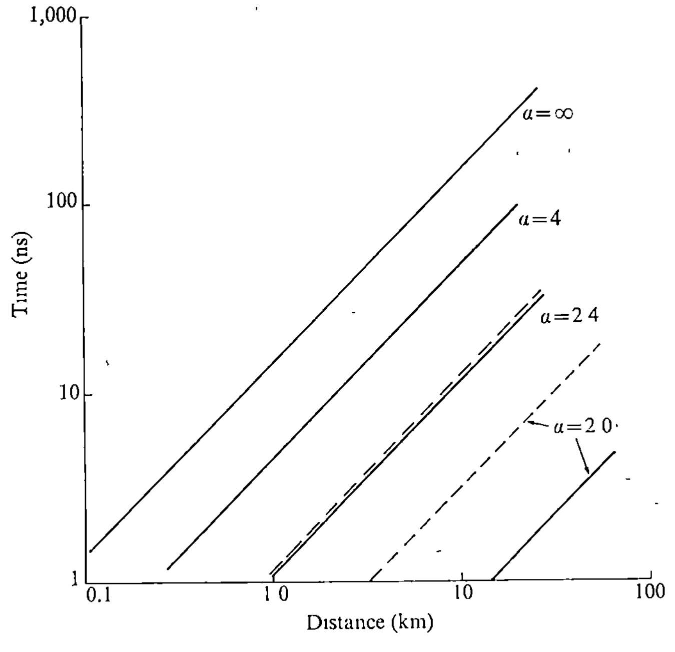
It is important to note one other factor that can seriously limit the performance of a fibre in reality, namely material dispersion. The figure quoted above of 0.18 ns km^{-1} for the mode dispersion in the fibre was not the measured total dispersion. The measured result was 0.25 ns km^{-1}, the difference being accounted for by the presence of dispersion from the material of the fibre itself, the dispersion that is used to good effect in prism spectrographs. The amount of pulse broadening from this effect depends on the linewidth of the source used. In the above case, a broadening of about 0.18 ns km^{-1} was due to this effect, corresponding to the use of source whose linewidth was approximately 1 nm. The dotted curves in Fig. 5 show the dispersion resulting when a source linewidth of 2 nm is used.

The presence of material dispersion thus sets a limitation on the choice of source, its linewidth being of paramount interest. For example a GaAs laser source with linewidth of 2 nm gives rise to material dispersion of approximately 0.3 ns km^{-1} whereas a GaAs LED with much greater linewidth, say 20 nm, generates material dispersion of 3 ns km^{-1}, limiting its use to only the lowest data rate systems. An interesting alternative lies, however, in an observation first made by Kapron and Keck that the material dispersion (governed by $d^2n/d\lambda^2$) passes through zero for silica, the most popular fibre material, in the region of 1.2–1.3 μm. This also corresponds to a favourable low loss transmission region in most fibres, but did not initially attract any interest because of the absence of both sources and detectors to operate there. Recent work on GaInAs LED sources suggests, however, that some reappraisal of the possibilities may be due, since operation in this spectral region would apparently make possible wide band optical systems using large core graded-index fibres with LED sources, thereby side-stepping many of the problems inherent in developing laser sources.

It is evident from the discussion of propagation in optical fibres that the design of cable for optical systems is likely to present some new problems. The most immediately obvious arises from the requirement that the fibre should not be subjected to microbending of the type likely to induce unwanted mode coupling. This means in practice that the fibre must be well cushioned within a small longitudinally rigid structure. An attractive theoretical design would be a rigid walled tube, filled with an easily compressed material in which the fibre was embedded, so that its natural stiffness allows it to lie straight within the spongy environment. Practical packaging to date involves extruded plastics chosen to balance cushioning, resistance to exterior forces and stiffness, in a single extruded coating, with nylon as a popular material. An alternative approach that is nearer to the theoretical optimum is the use of a polymer tube, in which the polymer molecules have been longitudinally aligned by stretching the tube after extrusion and within which the fibre lies loosely. The molecular alignment offers a tenfold increase in longitudinal Young's Modulus, generating a relatively very stiff tube while the loose fibre is free to flow over any irregularities that remain. Both approaches have led to reports of packaged fibre with no measurable (± 0.5 dB km^{-1}) change in loss from microbending caused by the packaging process.

Once the fibre has been successfully cushioned against its

Fig. 5 Theoretical dispersion curves for graded core fibres with different values of the parameter α. The dotted curves include material dispersion characteristic of a 2-nm linewidth source.



immediate environment, it must be enclosed into a cable designed to allow continuous lengths of order 1 km length to be pulled into an underground duct. This raises problems which take one into a new realm of glass technology. A sizeable amount of information is available on the strength of glass fibres. It shows that small diameter or short fibres are stronger than large or long ones, in terms the mean strain permissible before breakage occurs. A great deal of study has been devoted to the development of the theory of microcracks to explain the observed behaviour of glass samples. The characteristic of glass, well known to everyone, is that it deforms elastically to a certain strain level and then fails catastrophically. There is no plastic flow region as there is with metals. The strain that a given sample will take before failing depends critically on its surface state, since small or invisible cracks or defects in the surface lead to premature failure because of the stress concentration that occurs around them. The result is twofold. The surface of the sample must be protected as thoroughly as possible and there are a number of proprietary techniques for doing this. But recognising that no protection is likely to be perfect, it must be assumed that the effect of such protection will only be to reduce the probability of failure in any given length. Laboratory based measurements have been confined almost entirely to fibre samples of the order of 1 m long. Suddenly one is concerned with the failure statistics of fibre lengths that are longer by a factor of 10^4.

Extrapolation of the present data by four orders of magnitude is clearly likely to lead to misleading conclusions, so that experiments to test long lengths of fibre are being attempted, primarily aimed at verifying the existing statistical model developed by Weibull to describe such effects. A few tests of the failure probability for a given load applied for a short time have been made now on multi-kilometre lengths and the results shown to agree with the general predictions of Weibull. These were made by reeling long lengths of fibre from one reel to another under progressively increasing tension until breakages occurred.

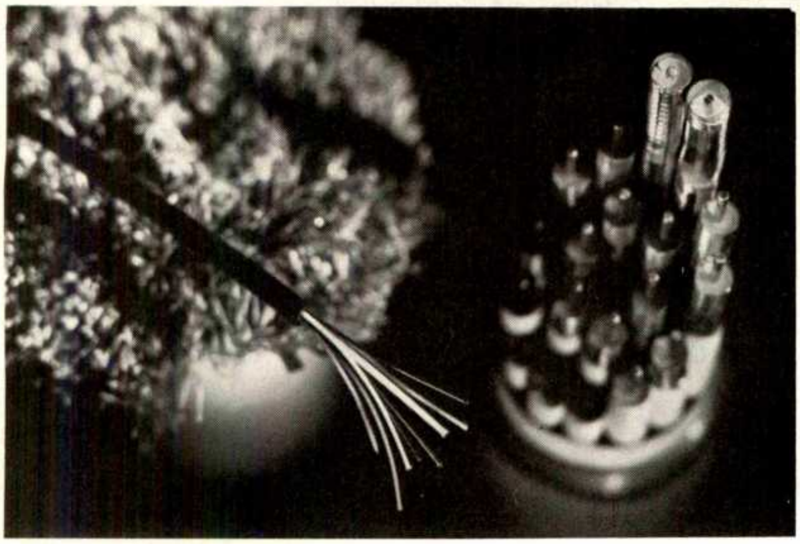
A second factor that requires further study is, however, the effect of microcrack growth in a glass filament when under a small but continuous strain. It is known that a fibre under a maintained strain well below its immediate failure level, will fail eventually because of the gradual growth of small cracks. Obviously, a fibre in a cable which has been pulled into a duct is likely to remain in a state of strain after the cable has been installed. At present it is not known what residual strain will be acceptable in the fibre to ensure an acceptably low failure rate over 10 or 20 years, although the initial indications suggest that strains of as low as 0.1% are the maximum allowable. To achieve such low levels of strain in cabled fibre, assuming that lengths of 0.5–1 km are to be pulled into ducts at a time, presents a new challenge to the cable designer. For comparison, we note that many present telecommunications cables can suffer strains of up to 10% in extreme cases without any major failure or serious degradation in their performance.

Having discussed some of the possibilities of this new transmission medium we shall now turn to the question of measurement techniques used to study and characterise it. It presents to the system engineer some interesting new problems not previously met in the study of existing transmission media. The most general point is that it presents in an acute form the problem that what you measure largely depends on how you measure it. To illustrate this cryptic comment, we refer back to the preceding discussion and consider how one should measure the pulse dispersion or bandwidth of a fibre transmission line and we compare this to a comparable measurement for a coaxial line. In the latter case, a fast (delta-function) pulse would be launched into the line at $L = 0$ from an impedance matched source and at distance $L = D$, a matched detector would record the received electrical signal which could then be analysed. Interpretation of the result might be complex, but the experimental result is unlikely to be very sensitive to the measurement situation.

In the case of the optical fibre, we can postulate a large number of possible measurements. We might take a short fibre length, and a single mode laser, launch a fast pulse into a single guided mode and detect the result before it had travelled far enough for significant amounts of energy to be coupled into other modes. We would have measured the very small dispersion associated with a single mode of the fibre. Or again, we might take a large area diffuse LED source, illuminate the whole end of the fibre exciting all modes, and again after a length short enough for there to be no mode mixing, we measure the pulse broadening. From this we can again deduce pulse dispersion, but now it will be a combination of mode dispersion and material dispersion, most probably dominated by the former. In either of the two former situations, we could allow propagation over a longer length so that mode coupling and mode dependent loss effects begin to play an effect. Ultimately a nearly stable mode distribution would be obtained, a distribution of energy among modes that once established will remain essentially constant. We can now remeasure the dispersion associated with the fibre. It will follow a curve of dispersion versus length of the general form shown in Fig. 4 with a linear initial growth, followed by a square root growth thereafter. Evidently, this is a much better measurement, provided we can clearly separate the linear and square-root regions.

Assuming this to be done in the laboratory, however, nagging questions remain. The last result depends critically on the amount of mode coupling present in the fibre. If the bare fibre is stored on a drum, this is sensitively dependent on the fibre tension, which controls how closely it follows microbumps on the reel surface. In a cable, similar considerations are likely to apply although the magnitude of any effect will evidently depend very much on the precise features of the cable design. We should also note that it is not only pulse dispersion that depends on the mode coupling and modal power distribution, but also the other critical design parameter, power loss against length. There is a unique loss rate (in dB km^{-1}) associated with a given stable mode distribution, but the region leading to that distribution may feature a higher or lower figure depending upon the launching characteristics. Both figures will change if the degree of mode coupling changes. In summary, we can say that there is not a single figure that can be quoted for either loss or pulse dispersion to characterise any particular multimode fibre. To understand the propagation in the fibre, a much more sophisticated model must be adopted, but out of this, guide rules will have to be distilled that can be used by the systems engineer, rules that will allow him to design a given link with confidence that sufficient margins will have been incorporated. In the short term, then, a great challenge is presented to optical measurement technology to thoroughly elucidate the details of transmission in the deceptively simple looking, yet very complex optical transmission line. A number of approaches are being developed.

It is evident from the above discussion that a measurement process must at least allow one to study propagation on very long lengths fibre, comparable to the lengths expected to be used in an operating system (say 10 km). But in addition, if the relative parts contributing to the overall dispersion and loss are to be resolved to allow design predictions to be made, measurements are necessary on intermediate lengths. The one technique that comes closest to achieving these requirements in a single step is the shuttle-pulse dispersion measurement, which is very reminiscent of echo measurements made on imperfectly terminated transmission lines. Experimentally a length of fibre, of perhaps 500 m or 1 km total length is mounted between high reflectivity mirrors with a source launching into one end and a detector at the other end. The launched pulse shuttles between mirrors, and the leakage power after every two transits is measured on the detector. By careful design of the apparatus and with low loss fibre, multiple transits can be observed, allowing a plot of dispersion against integer multiples of length to be made. Experimental curves



An optical fibre cable with, for comparisons of size, weight and raw material consumption, a 4,800-pair distribution cable (which carries local telephone traffic from junctions to individual receivers—maximum capacity 4,800 two-way circuits) and 18-core 9 mm tube coaxial cable, the largest currently used by the Post Office. This has a capacity of 90,000 voice circuits or 18 colour television signals. The optical cable capacity (with eight fibres operating at 140 MBit s^{-1} is 7,680 two-way circuits or eight colour television signals. It has expansion capacity at higher bit rates to surpass the coaxial cable capacity.

Standard Telephones and Cables

have been obtained in this way that closely follow the form shown in Fig. 4. In principle, this type of measurement could easily be used to study the effects of different launching and fibre lay conditions, so building up families of performance curves. This kind of measurement does not, however, go very far towards explaining why a fibre performs in a particular way. More penetrating measurements are required to achieve such an understanding. In particular, direct measurements of the mechanisms controlling the effects are needed if they are to be thoroughly understood, controlled and exploited.

The simplest study which throws some light on the underlying mechanisms of mode coupling in a multimode fibre is the measurement of the power distribution among the modes as a function of distance, with various excitation distributions. The reduced mode number M equates closely with the angle at which energy leaves the guide when it is terminated with a plane end and allowed to radiate into free space. The semi-angle θ of the cone of radiation from mode number M is given by

$$\theta = \frac{M\lambda}{4a} \tag{5}$$

where a is radius of the core. Thus in a large core fibre, in which the maximum value of M will be large, measurement of the distribution of optical power in the far field gives a simple estimate of the power as a function of mode number. If the fibre is excited by a single mode source coupling predominately to mode $M = 1$, then by cutting back the fibre, the spread of energy out through the mode spectrum can be studied. The measurement is unfortunately destructive but nevertheless does yield valuable information on the type of coupling present and its strength. A variation on this technique monitors the far field distribution while the launching distribution is changed, allowing rather more information to be obtained non-destructively.

A number of other techniques can be brought to bear on the problem, the most powerful potentially being those in which the energy scattered from the fibre is studied as a function of wavelength, angle or distance. Unfortunately such measurements are extremely difficult to make under well enough controlled conditions to obtain useful results. But a related approach promises to allow very sophisticated studies to be made of highly overmoded fibres, namely the recently developed and demonstrated capability to generate and detect very pure single LP modes in fibres capable of supporting tens, hundreds or thousands of such modes. The principle is essentially the same as that involved in launching pure single modes in planar guides (integrated optics) using a high index prism to couple energy to the fibre by tunnelling from an incident beam through the evanescent field of the cladding into the guided mode core field. Careful control of the angle of incidence and the incident beam profile allows efficient excitation of chosen guided modes of the fibre. This technique was used to obtain the mode shown in Fig. 2.

If the fibre under the prism is maintained parallel, then only modes with significant fields at the cladding–prism interface can be excited efficiently in practice. This means that only those modes close to cutoff can be studied. When the fibre is tapered down under the prism, modes that are well guided in the parallel fibre become accessible at different points along the prism face, so that the whole mode spectrum can be accessed. In either case, the technique can be reversed to allow the power being carried in different modes of the fibre to be separated and studied. For example, in this way the propagation constants of large numbers of discrete, identified modes in a multimode fibre can be measured and information about the index profile deduced. Use of these techniques also opens up the possibility of studying deliberate mode coupling between chosen mode pairs, and some preliminary work of this nature has been reported. The fibre was sandwiched between a pressure pad and a small amplitude grating fabricated on the surface of a sheet of plastic, so that the fibre lay was deformed sinusoidally in a controlled amount at a fixed spatial frequency. The effective frequency could be changed by rotating the grating about its normal axis relative to the fibre. Strong selective coupling was observed between pairs of modes according to the selection rules discussed earlier.

From the point of view of the engineer, enough progress has been made in the fibre and components to make possible serious system design[4]. Work is in progress in many laboratories assembling and studying prototype systems, most of which are based on the use of multimode large core diameter fibres. But there remain the finer points to be established, requiring more sophisticated measurement and interpretation of results and only from this second round of studies will the engineering data emerge that are needed for operating systems design. In the meantime the prototype engineering is posing challenges across a broad front to workers of many skills[5], from materials studies to optical measurement techniques and electromagnetic theory and is forcing a more critical appraisement to be made of previously simple models for fibre propagation.

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Integrated optical circuits for telecommunications

E. A. Ash*, C. W. Pitt* & M. G. F. Wilson*

Optical signals can be guided and manipulated in thin films deposited on a substrate. The new technology will allow the implementation of key circuit functions at optical frequencies.

THERE is no longer any doubt that optical fibre waveguides will shortly be used in telecommunication systems[1]. It is an advance which will have an increasing and, in our judgment, dominant role in the development of new communication systems for the remainder of this century. The fundamental advantages of an optical fibre as a communication medium compared for example with a coaxial line must, we feel, eventually prove decisive in many applications. These advantages include the bandwidth, which, at least in principle, is very large; the fact that the cost of the basic fibre material (silica) is negligible as compared with copper—and we are likely to run out of copper before we run out of sand; the almost total lack of interference between different optical fibres, in marked contrast to the situation with coaxial cables, where crosstalk can often represent a daunting problem; the fact that optical fibres are extremely economical of space, occupying an area which is less than one thousandth of that of an equally potent coaxial line.

The technological problems which must be solved before the total potential of optical fibres can be realised are formidable. For reasons which are more fully developed in the preceding article[1], the initial targets will be modest. The transmission medium will consist of fibres with relatively large core diameters (around 50 μm), capable of supporting a large number of optical modes. Since these modes all travel at somewhat different velocities such multimode fibres are rather dispersive, and this limits the bandwidth which can be achieved. Nevertheless it will suffice for the transmission of one or several colour television pictures or the equivalent amount of other forms of data. These early systems will also be restricted to a basic level of topological simplicity (Fig. 1), where a source, be it a laser- or light-emitting diode, is attached to the fibre at one end, and an optical detector at the other. One does not envisage any form of branching circuits. The system is in this sense comparable to the early forms of wired telegraphy over 100 years ago, where each wire had a key at one end and a detector at the other.

When the concept of optical fibres was first seriously discussed, a little over five years ago, it was suggested that perhaps one would never need anything more complex than the linear system of Fig. 1; that fibres would be so small, and so cheap, that one would always prefer to add additional fibres to a system, rather than elaborating the structure in each individual channel. In the intervening years it has become clear that the technological problems of making low loss, reproducible, low dispersion and robust fibres are not yielding without a fight. It is also clear that at least initially it will not prove easy to incorporate a very large number of fibres into a single cable. It is therefore by now accepted that every fibre in a cable will have to be cherished—that it is incumbent on the designers to make optimal use of each. One way in which one could make better use of a fibre than in the system of Fig. 1 is by using the same fibre for both "go" and "return" traffic. Clearly to do this, one must in some way split the channel into two sections at each end of the fibre. In principle this is readily accomplished using nothing more elaborate than a couple of semi-silvered mirrors as shown in Fig. 2. It is at this point that engineers who have some familiarity with the installation of communication circuits in the field will list some of the painful facts of their lives—such as manholes, into which the repeaters have to be inserted and which may be half full of water. The use of classical optical components needing careful alignment is not entirely ruled out—but very nearly so.

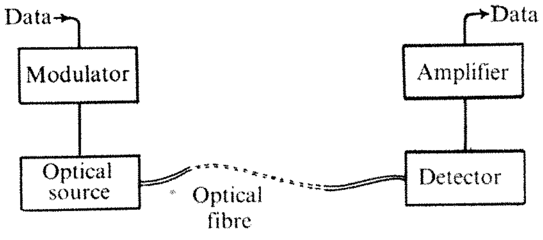
Fortunately we are witnessing the emergence of a new branch of optics—integrated optics[2]—which shows every sign of being able to perform not only such passive functions as required for the system of Fig. 2, but also a whole range of active functions such as generation, high speed modulation, switching and multiplexing. Integrated optics is lagging behind fibre optics by perhaps as much as a decade. The first generation of optical fibre communication systems will remain within the confines of the system of Fig. 1. But thereafter we see an increasingly important role for integrated optics, in allowing better use to be made of single fibres and in promoting the design of much more complicated and flexible systems. Our purpose in this paper is briefly to review the nature and current status of integrated optics, and then to indicate some of the ways in which it is likely to be incorporated in future optical communication systems.

Thin films

Integrated optics, alias, "thin film guided wave optics", is concerned with manipulating optical beams guided in a thin film deposited on a substrate having a somewhat lower refractive index. Provided that the film is sufficiently thick it can propagate one or more optical modes. It is also possible to confine such an optical mode laterally, for example by reducing the thickness of the film on either side of the guiding region (Fig. 3). For many purposes it suffices to guide vertically and permit some lateral diffraction of the beam. Typically the film thickness is of the order of 1 μm, a value which allows the use of many of the known techniques for thin film deposition.

Thin films are of course widely used in classical optics for anti-reflection or filter purposes. In these the light propagates normally to the plane of the thin film. In integrated optics propagation proceeds along the thin film. The losses

Fig. 1 Basic optical fibre communication system.



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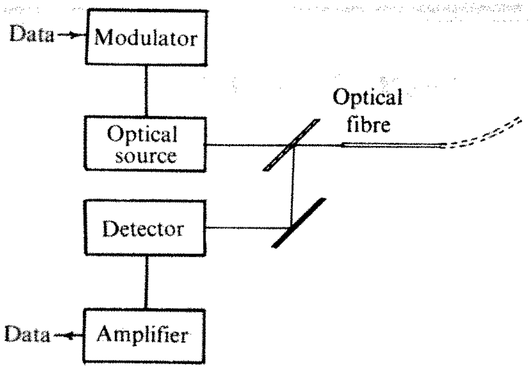


Fig. 2 Terminal for a 2-way optical fibre system, using mirrors for separation of go and return paths.

associated with such films are therefore a much more critical consideration. It has proved possible to achieve attenuation rates of less than 0.1 dB cm^{-1}. In many situations, however, one can work with attenuations of 1 dB cm^{-1} or even higher. These figures are perhaps five orders of magnitude worse than those required, and achieved, in the optical fibre. In integrated optics, however, one is primarily concerned with the loss per wavelength rather than the loss per km, the former figure representing some sort of "*Q* factor". It turns out that one can make very good components with materials where the fractional loss per wavelength is around 10^{-4}.

Before discussing the materials and other problems encountered in integrated optics, it may be helpful to indicate how one would translate the system of Fig. 2 into thin film form, Fig. 4. The role of the semi-silvered mirror is here played by a Bragg grating structure designed to give a 50 : 50 split. The laser and detector may be mounted on the optical substrate. It is, however, entirely possible that one would resort to the use of a material based on GaAs, in which case, the laser as well as the detector could be integrated into the structure itself. The overall size of the completed integrated circuit might be 4 mm square. Once made, the components are all rigidly disposed, and no adjustments in the field are required.

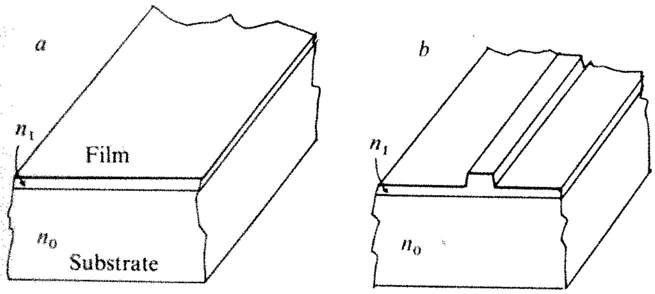
The feasibility of structures of this kind depends in the first instance on the availability of combinations of materials and technologies for depositing the films.

Materials and technology

The selection of materials and deposition technology depends to a large extent on the function of the optical components in the circuit; if devices with laterally defined geometry such as in Fig. 3*b* are required, the choice of shaping technology will impose further constraints.

The basis of an integrated optical device is light guiding within a thin film. This limits the choice to a film material having a higher index than its supporting substrate. The refractive index and thickness of the film will then determine the number and polarisation of modes propagating a transverse plane wave within such a planar guide. In classical optics terms, the refractive index condition ensures total internal reflection of light in the film, while the thickness limitation means that successive reflections of the wave from the thin film surfaces interfere constructively to allow energy to propagate.

Fig. 3 *a*, Basic integrated optical system. The refractive index n_1 of the film is greater than that of the substrate n_0. *b*, Integrated optical system showing one form of waveguide to prevent lateral diffraction effects.



A secondary requirement for such guides is an adequately low loss. This may vary from less than 0.1 dB cm^{-1} for an interconnection between components in the circuit, to several dB cm^{-1} for a short active component. Loss, resulting in signal attenuation, derives from three mechanisms: absorption of light in the material, scattering of energy from discontinuities in the refractive index of the film, and scattering from roughness of the film surfaces. The manifestation of these effects varies strongly with the material and the procedure adopted for film formation. For example, a dopant such as copper can be introduced by diffusion into a single crystal material such as magnesium oxide to form a waveguide; its effect will be to increase the real part of the refractive index but it will have little effect on the absorption (the imaginary part of the index). If, however, a few parts per million of copper are inadvertently introduced into a sputtered glass waveguide it results in severe absorption. The effect of bulk and surface perturbations of the index and topography of the guide depend on the magnitude of these effects in relation to the wavelength. Scattering of an electromagnetic wave from a randomly rough surface is negligible if glancing incidence occurs, provided that the wavelength is large compared with the roughness amplitude, or that the correlation length of the perturbation is greater than a few tens of wavelengths.

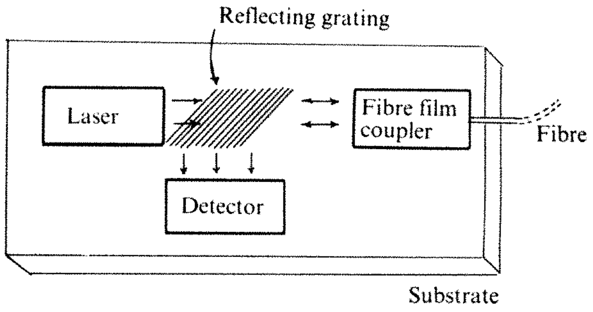


Fig. 4 Integrated form of two-way communication terminal.

These considerations of the material specifications bear on the basic problem of guiding; but we also need to manipulate the optical signal. Whereas some functions may be achieved with passive components, such as lenses, diverging guides and gratings, the realisation of a light source, modulation, switching or detection function requires the use of active materials. These may be lasing, electro-optic, piezoelectric, or photoelectric sections of the circuit. Such an additional degree of freedom for the circuit designer must, however, be paid for in an increased fabrication complexity. For example it may be desirable to phase modulate a signal in a zinc oxide film on a glass substrate. The electro-optic coefficients of ZnO are relatively large, producing a suitable change of refractive index when an electric field is applied across the guide. The appropriate crystal orientation (*c* axis parallel to the applied field) may be achieved by sputtering the material in appropriate conditions[3]: the film structure is polycrystalline . At typical deposition rates for such a film, the crystalline column dimensions are unfortunately similar to the wavelength of visible light, and prove to be efficient scatterers so that

Table 1 Materials and technology of integrated optics

Substrate material—refractive index and crystalline state	Film Material—refractive index and crystalline state	Operational wavelength range	Film deposition technique	Film shaping process	Guide attenuation dB cm^{-1}	Component class
Glass Soda-lime silicate glass $n_{0.63\mu m} = 1.5124$ Amorphous	Glass Aluminium borosilicate $n_{0.63\mu m} = 1.52$–1.58 Amorphous	Visible to near infrared	Sputtering	Ion etching	0.5–2.0	Passive
	Organics Photoresists, fatty acids, polymers. $n_{0.63\mu m} = 1.5$–1.6 Amorphous and planar ordered	Long ultraviolet to visible	Solvent deposition, Langmuir process, Chemical vapour phase.	Selective polymerisation, or scission (direct lithography by irradiation)	0.1–2.0	Passive
	Metal oxides for example Al_2O_3, Ta_2O_5, Nb_2O_5 $n_{0.63\mu m} = 1.60$–2.24 Amorphous and polycrystalline	Visible to near infrared	Electron-beam evaporation, Sputtering, Chemical vapour phase	Ion or chemical etching	~0.5–5.0	Passive and active
	Doped glass Ag substituted sodium glass $n_{0.63\mu m} = 1.6$ Amorphous	Ultraviolet to visible	Ion exchange	Masking of non-guiding region	~1.0	Passive
Crystal dielectric Lithium niobate $n_{0.63\mu m} = 2.20$ Single crystal	Doped crystal Titanium dopant $n_{0.63\mu m} = 2.22$–2.25 Single crystal	Visible and near infrared	Evaporation (of dopant) and diffusion	Confinement of diffused metal to guiding region	0.1–1.0	Active
	Titanium dopant $n = 2.25$ Partially disordered crystal	Visible and near infrared	Ion implantation	Confined ion-beam or masking	0.5–5.0?	Active
	Lithium depleted $n = 2.21$–2.22 Single crystal	Visible and near infrared	Out diffusion of lithium	Passive masking (to prevent egress of lithium)	1.0	Active
Semiconductor Gallium arsenide $n_{0.905\mu m} = 3.34$ Single crystal	Graded doped crystal GaAsP $n_{0.9\mu m} = 3.4$ Single crystal	Near infrared	Vapour phase epitaxy	Chemical or ion-etching	1–5	Active
	Proton irradiated GaAs $n = ?$ Disordered crystal	Near infrared	Ion implantation	Chemical or ion-etching	~8	Active
	Epitaxial multilayer GaAlAs/GaAs $n_{1.0\mu m} \approx 3.3$ Single crystal	Near infrared	Molecular beam epitaxy	—	~10	Active

the attenuation approaches 100 dB cm^{-1}. By careful modification of the process, the crystalline dimension may be reduced to rather less than the wavelength, with a corresponding reduction of the loss to about 8 dB cm^{-1}.

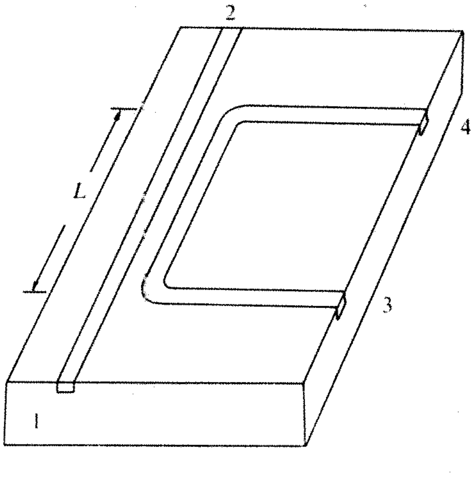
It is inevitable that the integrated optical circuit will introduce significant additional attenuation which it is important to minimise. One method of achieving this aim is to reduce the length of interconnection links in the circuit. This implies a capability to direct the light around small radius bends. Stripe waveguides meet these needs rather better than planar guides, but at the expense of an additional shaping operation, such as ion machining the planar films or restricting the film deposition to cover a predetermined path. Additional limitations are also imposed on material selection.

A prime requirement of a stripe guide is tight confinement of the energy within the stripe; unfortunately strong confinement implies a large index difference between the guide and surrounding media and this is the worst configuration for loss of energy from guide surface roughness. The introduction of two additional surfaces to the guide further degrades the attenuation due to scattering.

In the table we illustrate some typical material combinations with appropriate technologies for deposition and shaping. The technological selection problems may be illustrated by examining two of these systems which are currently used for fabrication of experimental devices.

The titanium doped lithium niobate[4] waveguide has found wide application because it combines a low optical loss with strong electro-optic and piezoelectric properties. The low absorption loss of the titanium ions may be attributed to their uniform dispersal throughout an almost defect free crystal lattice and the fact that they are in an oxidation state in which they exhibit small electron–photon interaction. Further, the waveguide is diffused resulting in reduction of scattering loss from the substrate–film interface (in contrast to a discrete film guide)—and also in the reduced edge acuity problems associated with defining the lateral boundaries of a stripe waveguide. The resulting waveguide has, however, a high refractive index and this leads

Fig. 5 Directional coupler; power inserted at arm 1 emerges from arms 2 and 4. The electro-optically controlled coupler incorporates electrodes on either side of guide 1–2 over the length *L*.



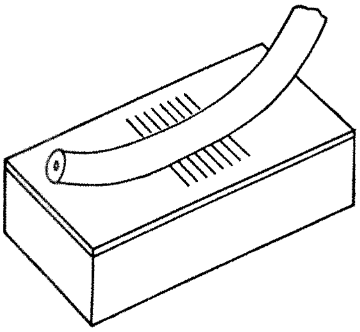


Fig. 6 Fibre–film coupler showing grating necessary for phase matching.

to a large scattering loss at the air–guide interface—which fortunately may be reduced by polishing. Less tractable is the problem of coupling guided energy inadvertently to the high refractive index substrate when a low refractive index medium, such as an optical fibre, is brought into contact to transfer energy to the fibre.

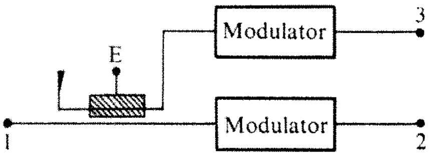
The GaAs material systems[5,6] are obviously attractive as they enable a lasing source to be interconnected through GaAs/GaAlAs waveguides to other active components made from the same materials and integrated on to one substrate. There are, however, two major problems. GaAs lasers operate at a wavelength close to the fundamental absorption edge of the material and proton-implanted or epitaxial GaAs guides exhibit a 'smeared' absorption edge resulting from damage or dopants within the guiding region. The broadening of the absorption edge extends into the lasing wavelength region resulting in the rather high absorption losses shown in the table. The other problem is similar to that encountered in lithium niobate—how to couple most of the laser energy from the high index GaAs circuit into low index fibre without incurring unwanted substrate interactions.

Functional elements

Although the name integrated optics accurately reflects the concept, little work has yet been done on integrating many components on to a single substrate. Research has concentrated on individual circuit elements, often derived directly from their counterparts in microwave engineering, whereas others resemble more closely components in bulk optics. A complete integrated optics circuit may include waveguides, power dividers, amplitude and phase modulators, switches, deflectors, filters, sources and detectors. To illustrate the current problems and state of the art, we shall briefly discuss three key components: directional couplers, fibre–film couplers and lasers.

A key component in any guided wave system is the directional coupler. It enables energy to be coupled, selectively, from one waveguide to another; its counterpart in conventional, free space optics is the partially reflecting mirror. The basis for directional coupling is the interaction of two waveguide modes having equal, or very nearly equal velocities. A particular configuration is shown in Fig. 5 where two waveguides are brought into close proximity. The fields of one guide overlap the adjacent guide; even though this overlap may be small, large transfers of power can occur over the interaction length L by virtue of the synchronism in velocities. Interaction lengths are of the order of 1,000 wavelengths so that a slight deviation from velocity synchronism will result in a large cumulative phase error between the coupled modes, leading to a serious degradation in performance. It follows that the tolerance restrictions on dimensions and material parameters are severe. Dimensional tolerances of less than 0.1% are typical. In spite of this, successful couplers have been fabricated using electron beam lithography. Furthermore, the tolerances can be relaxed considerably if the velocities are suitably tapered along the direction of propagation.

Fig. 7 Multiplexing circuit. Division of power from port 1 between ports 2 and 3 is controlled by the switchable directional coupler, terminal E.



Both the routing and modulation of optical signals can be performed with the help of electrically controlled switches. Several types of switch have been described which rely for their action on electroabsorption, Bragg diffraction and other well known processes; but in spite of different geometries most of them rely on a change of refractive index induced by electro-optic or acousto-optic effects. As an example, consider once again the directional coupler shown in Fig. 5. Suppose it to be fabricated in an electro-optic material with electrodes arranged so that an electric field can be applied across one of the coupled guides. With no applied field the coupler is designed so that all the power entering arm 1 is completely coupled into the adjacent guide and emerges from arm 4. When an electric field is applied to the electrodes the refractive index in one guide is changed, the mode synchronism destroyed and the power transfer reduced to a very small fraction, so that almost all of the input power emerges from arm 2. Although the obtainable changes in refractive index are small, of the order of 10^{-4}, the tolerance on synchronism is so severe that only a small change in index is sufficient to 'spoil' the interaction.

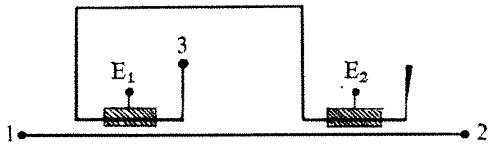


Fig. 8 Optical circuit for interconnecting any two of the three ports, 1,2,3 by means of two directional couplers, E_1, E_2.

Integrated optics will only play its full part in optical fibre communication systems when efficient fibre–film couplers become available. There are two main approaches to solving this problem. In the first the fibre is butt jointed to the end of the film. This method is the most direct and simplest in concept, but coupling is limited to the periphery of the circuit and the end faces of both film and fibre must be of high optical quality. The fibre must be aligned and held in position with respect to the film within a fraction of the core diameter. For efficient coupling the shape of the film and fibre fields should be matched as closely as possible.

To avoid some of these difficulties an alternative approach has been attempted: the fibre is pressed on to the film at any point on the circuit as shown in Fig. 6. Distributed coupling is achieved as in the directional couplers, the precise form of the fields is unimportant so long as overlap occurs. The grating shown in Fig. 6 is necessary to accommodate the possible large difference in velocity between fibre and film modes. The device is a directional coupler and the power transfer is determined by the interaction length and strength of coupling.

There is a wave velocity tolerance problem but again this can be overcome by suitable tapering. The fibre cladding must be partially removed to expose the core field to the film but this can be done by careful experimental techniques. A much more serious problem arises from the difference in index between film and fibre. This is especially marked when the substrate index is higher than the fibre

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Switchboard of the Royal Exchange Manchester, 1895.

index; it is this situation which is encountered when coupling a glass fibre to a doped lithium niobate film. It leads to a large fraction of the light being coupled to the substrate rather than to the film. Recent attempts to overcome this have relied on the deposition of even higher index materials on to the waveguide film but this brings us back to one of the original problems in integrated optics—the deposition of low loss high refractive index films. Thus the design of an efficient coupler is intimately bound to the materials problem.

Although most research work on semiconductor injection lasers proceeds independently of the work on integrated optics, there is a significant effort to produce thin film lasers which can be fabricated within an integrated optics thin film circuit. Consider the particular problem of fabricating thin film end reflectors. In the conventional injection laser these are provided by cleaving the end faces of the crystal. The integrated optics approach is to etch gratings having a pitch equal to one half wavelength of light so that the Bragg condition results in light being reflected normally from the grating array. The device thus comprises two sets of gratings on either side of the lasing film. In the distributed feedback laser the lasing action occurs within a single corrugated film obviating the need for two separate gratings.

It is interesting to note that integrated optics techniques can be used within the cavity of a conventional injection laser. This approach allows the use of a separate intracavity modulator which has significant advantages over direct laser modulation.

Integrated optics for fibre systems

We have already seen one example in Fig. 4 of an application of an integrated optical system permitting two-way communication along a single optical fibre. In this we assume that the transmitting laser at each end would be of the same frequency. The required 50 : 50 Bragg reflector in Fig. 4 implies a power loss of one half at each end, that is 6 dB, or three-quarters overall. It is a modest but not insignificant price to pay for being able to double the usage of the fibre. Since the operating wavelength of injection laser can be tightly controlled, one could also envisage a system in which the two lasers are separated by a small frequency interval. If the two laser frequencies differ, even by as little as 1%, it is possible to design the Bragg reflector so as to make it selective. It is then no longer necessary to incur this 6 dB loss.

Optical fibres have an optimum wavelength of operation, but in most cases this optimum is rather flat. Now the spectral width of injection lasers is very large as compared with other solid state or gas lasers, but is still typically no more than 0.2%. If the fibre performs adequately over a 20% frequency band, this would imply that one could have up to 100 separate frequencies propagating along the single fibre. The required channel dropping filters at each end of the fibre could be implemented by a series of tuned Bragg reflectors or using holographic couplers. Even if one were to implement this idea in a relatively modest way—perhaps 4 channels each way—it might well prove to be a better way of increasing the data capacity of the fibre than attempts to use very short pulses, which, quite apart from the dispersion problems, imply the need for very high speed electronics.

The inverse problem will also arise where the need is to feed several fibres from a single source as shown in Fig. 7. Here the power from the laser can be directed to either of the two fibres, or indeed split in some required proportion. Each channel will then be separately modulated by the electro-optic modulator. The same basic circuit can of course also be used to multiplex a single detector.

One can also anticipate situations where one would wish to interconnect three fibres, in such a way that any two can be interconnected. Figure 8, shows how this can be achieved using only two switchable directional couplers. The basic unit could of course be combined with others to realise more complex switching systems.

A final example is provided by the requirement for a data highway, where information can be injected on to or extracted from a fibre which may run throughout a large system—such as an aircraft or ship. Again the switchable directional coupler provides a solution.

The integrated optical solutions to these systems problems display a measure of elegance; the basic technology for their implementation exists. This might then raise the question as to why we anticipate that there will be a substantial delay before they are used in practice. Of course there is always a large gap between a technology which has passed a feasibility test, and one on which one can place the reliance which is essential in a telecommunications context. There is, however, an additional reason. As indicated by Midwinter[1], the first fibre systems will certainly use multimode fibre. Almost everything that we have said about integrated optical components and their application has tacitly assumed that we are concerned with a single mode optical fibre system. There is no doubt that single mode systems will arrive on the scene eventually—the technology is harder, but in every other way they are simply much better. We therefore foresee that the main applications for integrated optics will not make an impact until that time.

It would, however, be wrong to give the impression that integrated optics has no role in multi-mode systems. There are functions which can be performed even if one is faced simultaneously with a large number of modes. For example, we are currently developing switches and tapping points to be used in conjunction with multimode fibres. The attainable performance will fall short of that which can be anticipated for single mode systems but we nevertheless expect the devices to be of use. For the dawn of the golden age of integrated optics, however, we must await the arrival of single mode optical fibre systems.

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articles

r-Process nucleosynthesis of superheavy nuclei and nuclear mass tables

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New predictions of nuclear masses and fission barriers indicate that it is not possible to form superheavy nuclei during r-process nucleosynthesis.

THE r process is the sequence of rapid neutron captures and subsequent β decays responsible for the build up of many of the heavier nuclei in nature[1]. In particular, the r process is probably the only natural mechanism for the formation of nuclei with $Z \geqslant 83$ and $A \geqslant 210$. Because of this, there has been considerable speculation concerning the possibility of forming superheavy nuclei ($A \simeq 290$–300) in the r-process environment[2-10]. Here we summarise the results of an intercomparison of the r-process paths predicted by several of the more recent formulations for nuclear masses far from the line of β stability. These predictions include detailed empirical systematics based on nuclear decay energies[11], and the addition of higher order terms to the liquid-drop mass formula important for r-process nuclei (ref. 12, and W. D. Myers, unpublished). In addition to being the most up-to-date masses available, these masses have been derived by independent methods; on the one hand we have strictly empirical extrapolations, and on the other hand, predictions based on models with a minimum number of free parameters. Because these methods give the two extremes of nuclear stability, we believe that these mass tables should bracket the actual r-process nuclear masses. We find that the newer nuclear masses consistently preclude the possibility of forming superheavy nuclei during most of the r process.

r Process and nuclear masses

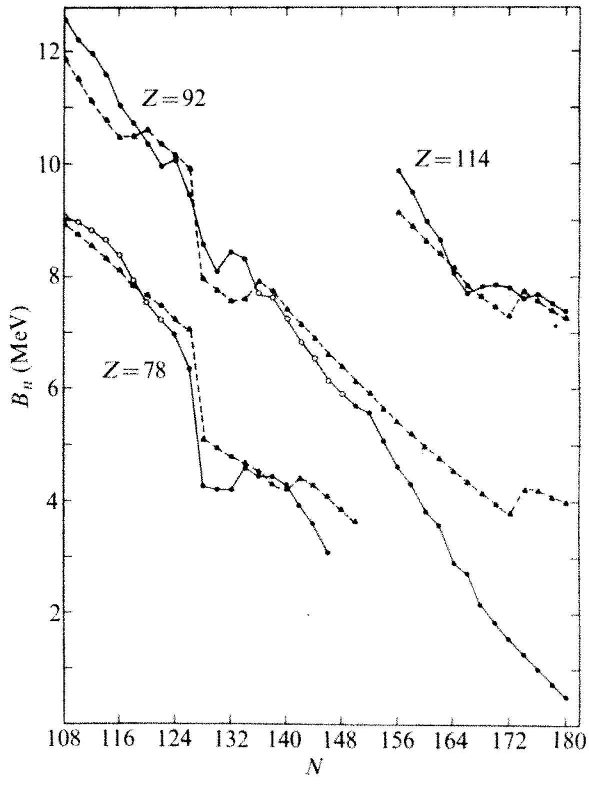
Nuclear masses enter into a calculation of the r process in two important ways. First of all, the neutron binding energies are used in the determination of the r-process path. In a static r-process calculation[2] one assumes an $(n,\gamma) \leftrightharpoons (\gamma, n)$ equilibrium. The high neutron flux responsible for this equilibrium presumably arises from violent stellar processes such as a supernova. The Saha equation[13] can then be applied to describe the statistical equilibrium between neutron emission and capture. Thus, the relative isotopic abundances are given by

$$\frac{\eta_n n(Z,A-1)}{n(Z,A)} = \frac{(2\pi\mu kT)^{3/2}}{h^3}\left[\frac{G_n G(Z,A-1)}{G(Z,A)}\right]\exp\left[\frac{-B_n(Z,A)}{kT}\right]$$

where η_n is the neutron number density; $n(Z,A)$ is the relative abundance of isotope A with atomic number Z; $G(Z,A)$ is the partition function for the nucleus ${}^AZ$; T is the r-process temperature, and $B_n(Z,A)$ is the neutron binding energy for the nucleus ${}^AZ$. The exponential makes the r-process abundances very sensitive to neutron-binding energies. As a consequence, one obtains a maximum r-process abundance path characteristic of each set of nuclear masses (see Figs 3–5). This path is centred along some optimum value of the neutron binding energy (typically ~3 MeV), and relative abundances become insignificant within several nucleon numbers of this optimum.

A second important influence of nuclear masses on an r-process calculation arises when one attempts to determine the values of Z and A where neutron induced fission begins to dominate neutron capture. This is the point at which the build up of heavier elements in the r process ceases. It has been shown[8] that, to a good approximation for r-process purposes, all nuclei can be said to undergo neutron induced fission whenever the sum of the incident neutron kinetic energy and the neutron binding energy exceed the height of the fission barrier. The determination of the (n,f) cutoff is then reduced

Fig. 1 Comparison between empirical and mass formula neutron binding energies for $Z = 78$, 92 and 114. It is seen that the empirical estimates tend to predict less stability for neutron-excess isotopes. The neutron binding energies for the Seeger and Howard masses (not shown) exhibit behaviour similar to the Myers and Swiatecki masses. ○, Measured values[24]; ●, ref. 11; ▲, Myers (unpublished).



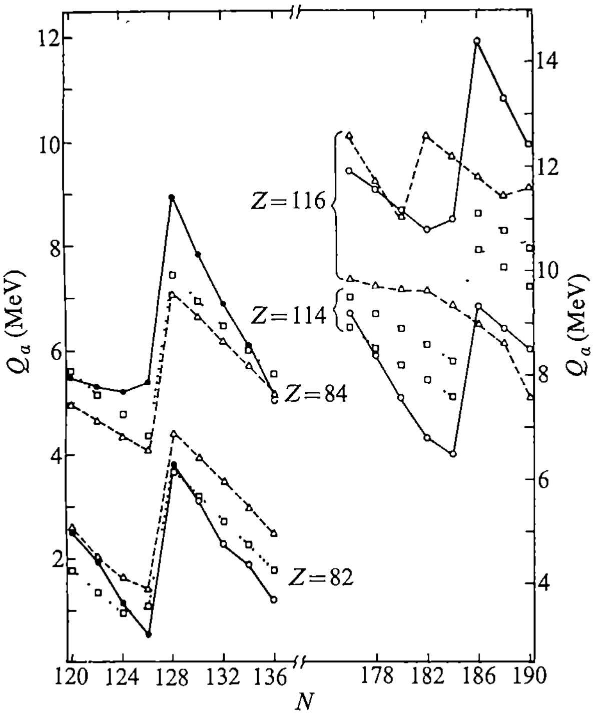


Fig. 2 Comparison between empirical and mass formula α-decay energies. It is seen that the effects of shell closures are more dramatic for the empirical masses . . . . , Myers (unpublished); - - - -, ref. 12; ——, ref. 11.

to the estimation of fission barriers and neutron binding energies, both of which are sensitive to the mass formula used.

Nuclear masses

We have used three different compilations of nuclear masses. Empirical estimates of the binding energies of r-process nuclei have been derived by Viola *et al.*[11] on the basis of α- and β-decay energy systematics. Since these are rather smooth except near closed shells and since the experimentally known α-decay chains span a rather broad range of nuclei, one can make extrapolations into the r-process region with some degree of confidence. Predictions based on α-decay systematics have been shown, for example, to yield rather impressive agreement with experimental α-decay Q-values of neutron-deficient nuclei[14].

The Seeger and Howard mass formula[12] involves the usual separation into a smooth macroscopic liquid drop term and microscopic corrections. The expression for the macroscopic energy includes shape-dependent surface and surface asymmetry terms, as well as a shape-dependent Coulomb term corrected for a diffuse nuclear surface. The microscopic corrections employ the Strutinsky renormalisation procedure and BCS pairing theory. The parameters of the formula are adjusted to give, simultaneously, self-consistent nuclear masses and fission barriers. The fission barriers are calculated assuming the idealised liquid drop saddle point shapes of Cohen and Swiatecki[15].

The third formulation of nuclear masses is the droplet model formula of Myers and Swiatecki[16]. In addition to containing many of the features of the liquid drop approach, the droplet model allows the neutron and proton densities to vary independently, and includes higher order terms in $(N-Z)$ which are important for the neutron-excess r-process nuclei. The new droplet model parameters, as in ref. 12, are determined from a fit to experimental masses and fission barriers.

In general the masses derived from empirical systematics predict increased stability for neutron-deficient isotopes and decreased stability for neutron-excess isotopes compared with the predictions of the mass formulae. This is demonstrated in Fig. 1 where we compare trends in neutron binding energies for $Z = 78$, 92 and 114. The binding energies for both mass formulae show similar behaviour. As far as the r process is concerned, this means that the r-process path for the masses based on empirical systematics will follow a less neutron-rich path than those of the mass formulae.

Also, the masses based on empirical systematics predict much stronger discontinuities in nuclear structure in the region of the closed shells. For example, in Fig. 2 we compare the α-decay Q-values for even–even nuclei near the $Z = 82$, $N = 126$ doubly closed shell. It is observed that the mass formulae predict much weaker shell strengths than are actually observed. This tendency for the mass formulae to underestimate shell corrections is an important consideration in the decay-back process after r-process freeze out. It implies that real nuclei just beyond the $N = 184$ closed shell should be more unstable than the mass formulae predictions for both α decay and, more particularly, spontaneous fission. Since many of the progenitors of the superheavy nuclei that one might expect to observe in nature must β decay through $N > 184$ species to reach the 'island of stability', this factor will further inhibit survival in the decay-back process.

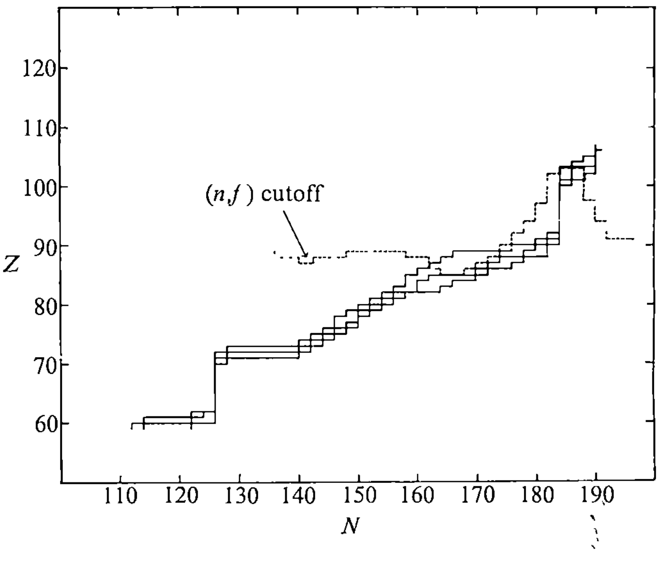
Results of calculation

To compare the different sets of nuclear masses we have performed a static r-process calculation, as outlined in ref. 2, for each mass table separately. The r-process temperature and density (or equivalently the r-process cycle time[2]) were then adjusted to reproduce the centroid of the experimentally observed $A = 195$ abundance peak. β-decay rates were estimated using the formulation of Senbetu[17] for calculation of f and assuming[6] an average log ft value of 6.5.

Figures 3–5 show the r-process paths, conditions, and the neutron-induced cutoff line estimated on the previously mentioned procedures. The path which best fits the $A = 195$ abundance peak is given by the central solid line on each figure. The paths on either side of the 'best fit' line correspond to a displacement of the centroid of the $A = 195$ peak by one mass unit. This was taken as the limit of uncertainty in the calculation.

Figure 3 shows the r-process path predicted for the Myers droplet model masses. We find that the 'best fit' r-process path crosses the (n,f) cutoff line at $Z = 85$ and $N = 164$. Although it appears at first glance that one might, with a slightly more neutron-excess path, temporarily escape the neutron-induced fission cutoff until $Z = 102$, this is actually not the case. As has been pointed out by Schramm and Fiset[8], relative abundances of a fission-unstable isotope as low as $\sim 10^{-10}$ are sufficient to

Fig. 3 r-Process path and fission cutoff for the Myers (unpublished) mass formula, using $T = 1.8 \times 10^9$ K, and $\eta_n = 1.5 \times 10^{26}$, 6.0×10^{26} and 2.2×10^{27} n cm^{-3}.



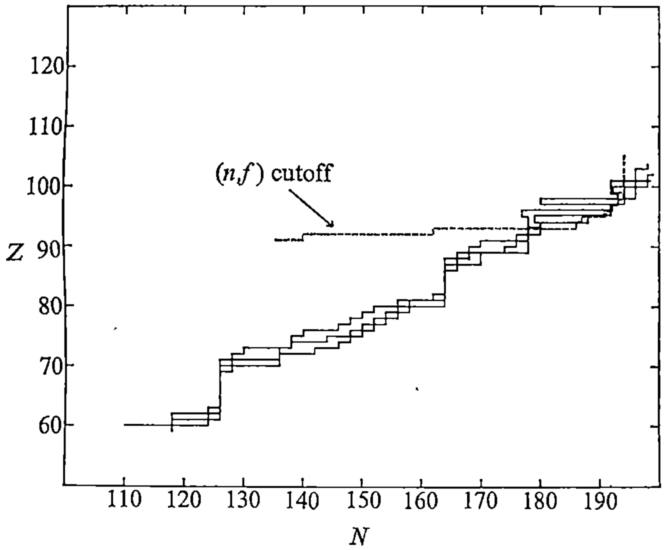


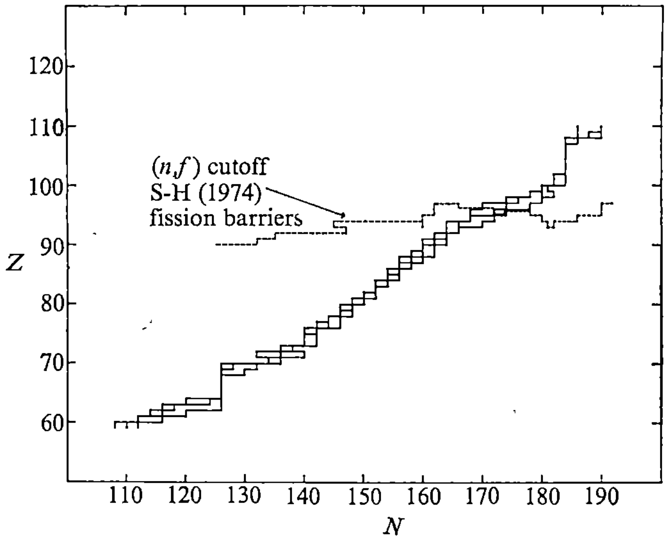
Fig. 4 r-Process path and fission cutoff for the Seeger and Howard mass formula[12], using $T = 1.8\times10^9$ K and $\eta_n = 1.9\times10^{27}$, 7.5×10^{27} and 3.4×10^{28} n cm^{-3}.

terminate the r process. This is so because the neutron capture rate, and thus the (n,f) rate, is so much more rapid than the β-decay rate: for example $\lambda_\beta/\lambda_n \simeq 10^{-10}$ for $T \simeq 10^9$ K, $\eta_n \simeq 10^{28}$ ncm^{-3}. To reduce the abundances of isotopes along the (n,f) cutoff line sufficiently, an extremely neutron-excess path is required, which we feel is outside the limits of uncertainty for the present r-process calculation.

Figure 4 shows the r-process path predicted for the masses of ref. 12. Here we find that the (n,f) line is crossed by the best fit r-process path at $Z = 93$ and $N = 178$, again short of the superheavy regime.

In Fig. 5 the r-process path predicted by the empirical masses of Viola *et al.*[11] is plotted. For our calculations using these masses, we took the fission barriers from ref. 12 since these give the best fit to experimental barriers. The empirical α-decay systematics do not extend below $Z = 50$, and the r-process calculations generally extend to $Z \simeq 40$; therefore, the Seeger and Howard masses were used to fill in the r-process calculation for the empirical masses. The results were not particularly sensitive to the choice of masses below $Z = 50$, since all of the mass formulae seem to predict similar behaviour in this region.

Fig. 5 r-Process path for empirical masses of Viola *et al.*[11]. The fission barriers for the (n,f) cutoff line are assumed from the tables of Seeger and Howard[12]. $T = 1.8\times10^9$ K; $\eta_n = 3.3\times10^{27}$, 1.9×10^{28} and 3.3×10^{29} n cm^{-3}.



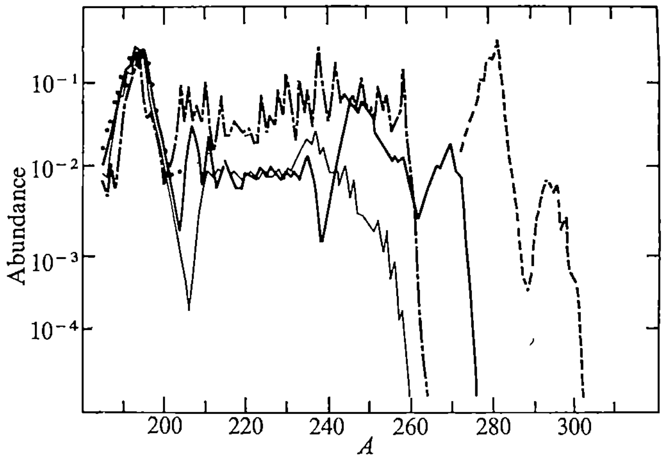


Fig. 6 r-Process abundances before decay-back predicted for the best fit to the $A = 195$ abundance peak for each mass formula. . . . , r-Process abundances; ——, Myers (unpublished); —— ref. 12; — - — - —, ref. 11, - - - - - -, ref. 16.

The abundances before decay-back of the r-process paths which best fit the $A = 195$ peak for each mass table are shown in Fig. 6. Also shown for comparison are the abundances calculated with the Myers and Swiatecki liquid-drop formula[16]. For this mass formula, a maximum Z of 100 before fission termination was assumed from the work of Schramm and Fiset[8]. Although previous work seemed to indicate that the r process could form the minimum mass number necessary to reach the island of superheavy nuclei[6], the newer atomic mass compilations provide a much more pessimistic conclusion.

The primary reason for the difficulty in reaching the superheavy nuclei in our calculation is the increased magnitude of the surface asymmetry term in the newer mass formulae. The importance of this term in r-process calculations has been pointed out by a number of authors[5,8,10]. This increase in the magnitude of the surface asymmetry term results in lower fission barriers along the r-process path and, hence, neutron-induced fission becomes important at lower values of Z and A. A secondary reason for the decrease in the maximum mass number achievable is that the newer mass tables predict slightly less neutron-excess r-process paths near the fission cutoff. This effect is most significant for the empirical masses, and is consistent with the predictions based on the energy-density mass formula of Brueckner *et al.*[9]. A consequence of these less neutron-excess r-process paths is that even if fission barriers were not reduced to the extent implied by the newer mass formulae, one would still encounter difficulty in obtaining mass numbers of $A \gtrsim 290$ needed to form the superheavy nuclei.

The fission cutoffs shown in Figs 3–5 might be considered as upper limits rather than actual limits for the following reasons:

(1) We have not considered here the problem of zero point motion which could tend to lower the effective fission barriers as pointed out by Schramm and Fiset[8].

(2) We have treated the neutron kinetic energies as a delta function at 100 keV. In reality, as pointed out by Johns and Reeves[18], one would expect a Maxwell–Boltzmann kinetic energy distribution for the neutrons. This would result in a large number of higher energy neutrons which could induce fission earlier in the r process.

(3) In these calculations we have not introduced a correction to the Coulomb energy from the interaction between the nucleus and the electronic charge in a dense stellar plasma. It has been estimated that this interaction should significantly reduce the effective fission barriers for r-process nuclei[19].

Conclusion

It seems that the most recent nuclear mass compilations make the possibility of the formation of superheavy nuclei during most of the r-process quite difficult. Although in a dynamic calculation one might expect to form a small amount of superheavy nuclei during 'freeze out' (ref. 20 and O. Johns, unpublished) the bulk of the r-process period (responsible for the $A = 195$ abundance peak) seems not to produce superheavy nuclei, according to the most recent predictions of nuclear stability. This is then, perhaps, one reason why experimental searches have yielded rather stringent upper limits on the abundances of superheavy nuclei in nature[21–23].

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Environmental and climatic implications of late Quaternary lake-level fluctuations in Africa

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Widespread water balance shifts in closed basin lakes reveal major glacial–interglacial variations in rainfall and streamflow. Markedly arid conditions characterising intertropical Africa during the last glacial maximum were followed by an early Holocene lacustral phase in which annual precipitation between 24 °N and 8 °S exceeded 150% of the 1931–60 mean.

We have compiled published lake-level data from 58 African basins in a simple cartographic form which emphasises regional patterns of surface water availability over the past 21,000 yr. With certain reservations, these maps can be used to interpret past precipitation–evaporation patterns. We hope that our approach will stimulate discussion on the long term shifts in atmospheric wind systems and moisture transport over Africa and surrounding oceans. The results also relate directly to past erosion rates and geochemical cycling, to the timing of aquifer recharge, to the distribution and spread of flora and fauna, and to the diffusion of prehistoric cultures over the continent. We suggest that they will explain many anomalies in the patterns of water-borne diseases. Since significant parallels exist with the lake-level chronologies of other regions, we hope to collaborate with other workers in extending this series of maps to other continents.

Africa has two advantages for this initial survey: a large land area spanning over 60° of latitude, where direct hydrological effects of glaciation were minimal; and the best coverage of radiometrically-dated studies of lake levels[1–7]. It has long been the scene of a lively debate on the question of the synchronism of glacial and 'pluvial' periods[8]. Changes in the water level have been inferred not only from former strandlines and overflow channels, but also from the depositional history of cores, exposed sections and archaeological excavations.

Technique

Relying exclusively on radiocarbon-dated chronologies, we have constructed a series of maps representing lake-level patterns during each millennium since 21,000 yr b.p. Selected examples appear in Figs 1–5. We have specifically excluded all relative dates, all archaeological information unrelated to shorelines, and inferences about past climates derived from regional vegetation or slope processes. Evidence from alluvial terrace sequences has not been plotted. We have also avoided lakes which developed by blockage of major throughflowing rivers like the Niger and the Nile.

In Figures 1–5 we use the tripartite division of lake-level curves devised by Butzer *et al.*[2]. Note that each basin is considered independently. Its datum level is its long term minimum water level and not the present value. The maps record the occurrence of high or intermediate levels in each basin within each 1,000 yr period. Millennia characterised throughout by minimal water levels are shown by open triangles. This method underemphasises brief arid periods, but makes best use of isolated ^{14}C dates, since datable materials are concentrated in lacustrine sediments. For cartographic reasons, basins < 100 km apart have been grouped together.

The reliability of these maps rests ultimately on the 250 internally and stratigraphically consistent ^{14}C dates on which individual chronologies are based; 79.6% are derived from lake limestone, mollusc and ostracod shells, or other inorganic carbonates, and 12.7% relate to disseminated organic matter or plant macrofossils, and the remainder to charcoal or bone. A large proportion are therefore liable in some degree to the contamination problems besetting continental carbonates[9]. Because of continuing uncertainties about the corrections for variations in ^{14}C production[10] and initial ^{14}C/^{12}C ratios in lake waters[9], this study is based on uncorrected dates. This is our main reason for choosing the rather coarse 1,000-yr interval for the maps. We feel that the consistency of the results, in spite of the present spate of new evidence, argues forcibly for the general reliability of the dating framework.

Regional shifts in lacustral conditions

Figure 1 shows the lake-level situation now: the map for the past 1,000 yr is not identical. The distribution of data points is

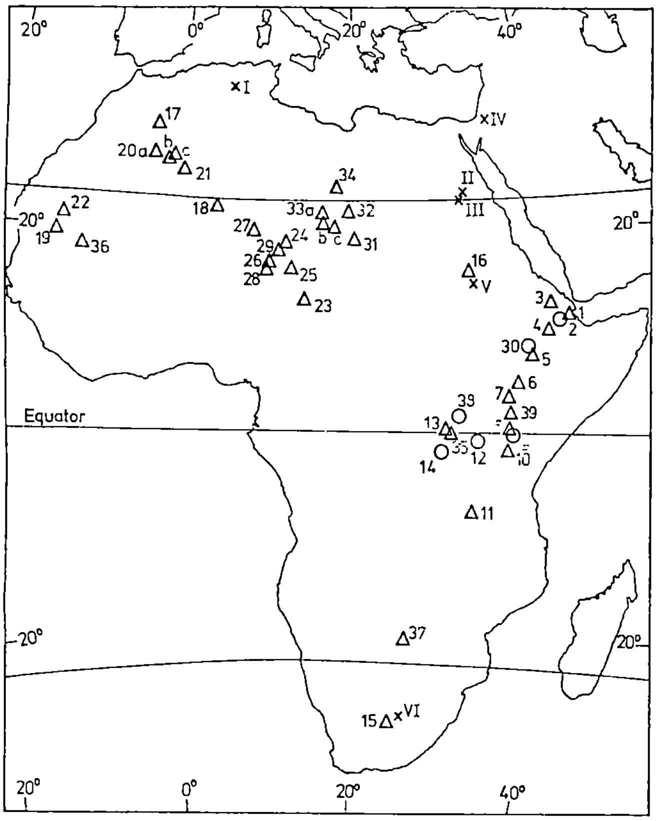


Fig. 1 Present day pattern of African lake levels △, low lake levels; ○, intermediate lake levels; ●, high lake levels. **Lake Basins**: 1. Assal[4,14]; 2. Lower Awash (Abbé and adjacent grabens) (Gasse, personal communication; and refs 4,5); 3. Afrera[5,13]; 4. Middle Awash (Besaka and Gawani) (Williams, personal communication; and ref. 86); 5. Ziway-Shala[12,37,88]; 6. Stefanie[12]; 7. Rudolf[2]; 8. Nakuru-Elmenteita[2,89]; 9. Naivasha[2,84,89]; 10. Magadi[2]; 11. Rukwa[2]; 12. Victoria[30,90]; 13. Katwe[40]; 14. Kivu[31]; 15. Alexandersfontein[25]; 16. Jebel Aulia[33]; 17. Wadi Saoura, Grand Erg Occidental[22]; 18. Hoggar[34]; 19. Khat Depression[91]; 20. Erg Chech-Touat[22] (*a*) Kadda, (*b*) Bou Bernous, (*c*) Bou Ali, Touat; 21. Ahnet-Mouydir Basin[22]; 22. Chemchane, Oum Arouaba, Aderg[15]; 23. Chad[6,41]; 24. Kafra, Bilma, Achegour[6,38,92]; 25. Agadem[6,92]; 26. Termit Ouest, Kandel-Bouzou[6,92]; 27. Adrar Bous[92]; 28. Bouloum Gana[92]; 29 Fachi[6,38,92]; 30. Wonchi[42]; 31. Ounianga Kebir[37]; 32. Mouskorbé[37]; 33. Western Tibesti[37] (*a*) Bardagué-Yebbigué, (*b*) Bégour, Trou au Natron, (*c*) Tarso Yega; 34 .Serir Tibesti[39]; 35. George[90]; 36 Dhar Tichitt[23]; 37. Makarikari (ref. 24 and Grove, unpublished); 38. Albert[31]; 39. Suguta (Bishop, personal communication).

Other localities referred to in text (×)

I Biskra; II Kurkur; III Dungul; IV Dead Sea; V Gezira; VI Florisbad.

patchy, since former lakes are clustered along the African rift valleys and in the closed basins of the Sahara. Suitable basins seem to be lacking within the integrated drainages of the Zaire and Zambezi. But unfortunate gaps exist in present knowledge of southern Africa, the eastern Sahara, and the north African chott region. The effect of volcanic or tectonic activity on rift valley lakes has often been questioned. Careful studies show that lake levels have rarely been significantly disturbed during the past 20,000 yr (refs 11, 12). Even in the particularly unstable Afar Depression, lake-level changes agree remarkably with the rest of tropical Africa[13,14].

Figure 1 implies that the present day is a relatively arid period. The few cores available from basins still occupied by lakes (areas 2, 8–9, 12, 14, 38) reveal the occurrence of much greater aridity in intertropical Africa between 21,000 and 12,500 yr b.p.

Figure 2 is a composite map covering the culmination of the last glaciation, 18,000±3,000 yr b.p. Data for this period are still sparse. We have not plotted any arid intervals which are unbracketed by ^{14}C dates. A belt of very low lake levels extended from Mauritania across the southern Sahara to the eastern rift. This followed a long period of intermittent highstand in tropical Africa[3,15,16] and Arabia (Vita-Finzi, personal communication) which has yielded radiocarbon dates $\geqslant$ 21,000 yr b.p. (Fig. 6). The level of Lake Abbé began to fall after 23,000 yr b.p., but did not reach its minimum till 17,000 yr b.p. (Gasse, personal communication). Its behaviour closely resembles the most recent data from the Dead Sea[17]. Because Fig. 2 simply records the presence of more or less surface water, it should not be used to infer times of transition.

Supporting evidence for widespread tropical aridity at the glacial maximum is summarised by Williams[18]. Deflation of desiccated lake sediments and soils readily accounts for the high frequency of freshwater diatoms and opal phytoliths discovered in core K8–57 off the West African coast[19].

The poleward fringes of Africa also experienced lacustral conditions before 21,000 yr b.p., but these persisted intermittently through the next 6,000 yr. Ponding of the southward drainage from the Algerian High Atlas (area 17) created an extensive terrace in which lacustrine levels are dated from > 39,000 to 30,000 and from 24,000 to 14,500 yr b.p. Higher groundwater tables are indicated about 22,900 yr ago in the Western Desert of Egypt[20] (localities II and III, Fig. 1) and around 19,000 yr b.p. near Biskra[21]. Significant recharge of aquifers in Tunisia and Algeria continued during the period 21,000–15,000 yr b.p. (refs 22, 23).

Information from southern Africa is still extremely limited. A very large freshwater lake occupied the Makarikari Depression[24] at 20,990 yr b.p. (GaK 4310), and lacustral conditions apparently prevailed in South Africa until 16,000 yr ago[25]. Higher ground water tables from > 32,000 to 14,000 yr b.p. are suggested by widespread spring and cave tufas on both north-west and south-east margins of the Kalahari[26,27] and by the record provided by spring vent activity at Florisbad[28,29].

Maximum aridity

Between 15,000 and 13,000 yr b.p. aridity became even more intense and widespread. Every documented basin in Africa experienced minimum water levels in the fourteenth millennium b.p., with the doubtful exception of Tibesti (Fig. 6). The small fluctuation between 14,500 and 15,000 yr b.p. is probably not real: 4 of the 5 constituent dates come from localities strongly influenced by hot springs (areas 5, 14, 33*b*). For at least 2,000 yr before 12,500 yr b.p., Lake Victoria, and probably also Lake Albert, ceased to overflow into the Nile[30,31]. Evaporites were precipitated in Victoria[30] and along the White Nile, which "dwindled to a mere seasonal trickle" in the central Sudan[32].

Shortly before 12,000 yr b.p. the long arid phase was interrupted in tropical Africa. The Ugandan lakes all regained their overflow levels[30,31]. Many other basins registered moderate shifts in water balance (Fig. 3). Increasing flooding in both White and Blue Niles gave rise to extensive lakes and swamps in the Gezira (ref. 33; M. A. J. Williams, personal communication, 1975). Downstream from Khartoum, the terrace sequence of the Nile reflects variations in load–discharge relationships, but their interpretation in climatic terms is difficult and some interpretations conflict with this model[2,32]. Arid conditions still persisted in the north-west Sahara, although small lakes or marshes developed as far north as the Hoggar massif[34]. These conditions lasted until about 10,500 b.p., when there was a brief drop in lake level in some tropical basins. We suggest that the period 12,500–10,000 yr b.p. is the African counterpart of the late glacial.

Between 10,000 and 9,000 yr b.p., an abrupt change of mode in Arabia (Vita-Finzi, personal communication) and tropical Africa marks the switchover to full interglacial conditions. 18 out of 20 data points suddenly record maximum lake levels. The striking peak at 9,500–9,000 yr b.p. in Fig. 6 is dominated by basal shell dates from thick lacustrine sequences. There is as yet no evidence that the north-west Saharan basins were affected before 8,900 yr b.p., although the South Basin of the Dead Sea began to overflow around 9,850 yr b.p. (ref. 35). Limited pollen data suggest that conditions in South Africa became warmer and drier[36] about this time.

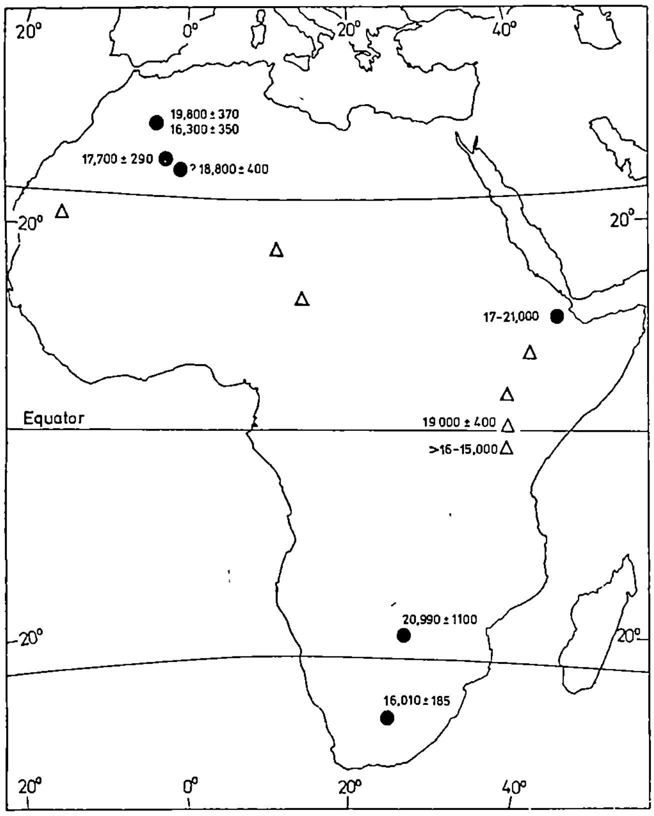
This early Holocene lacustral phase culminated between 8,000 and 9,000 yr b.p., when 31 basins experienced high stands (Fig. 4). The belt of high lake levels extended from Lake Rukwa (8°S 33°E) to the Hoggar (24°N 6°E) and Mauritania (21°N 13°W). Permanent water again existed along the Wadi Saoura. Renewed accretion of lake and swamp deposits took place between the Niles in the Gezira, and the central Saharan massifs supplied significant runoff to surrounding lowlands[37].

Lake-level record since 8,000 yr b.p.

Figure 6 shows a more complex behaviour since 8,000 yr b.p. Many basins experienced periods of low levels after 7,500 yr b.p. and did not recover until just after 6,500–6,000 yr b.p. The lesser rise which followed lasted until about 4,800 yr b.p. It was most pronounced in basins fed by groundwater in north-western and central Sahara[6,15,22,38] and the Afar[5,13], in Lake Chad, and in lakes maintained by runoff from the Ethiopian highlands (areas 5–7). Runoff from Tibesti again sustained lakes in the southern desert of Libya[39]. This rise, which was barely recorded in very marginal basins, marks the end of the Holocene lacustral phase in many areas.

The succeeding 5 millennia have seen a general reduction in surface water storage, punctuated by brief periods of greater runoff which were increasingly restricted in extent. We believe that the peaks in Fig. 6 are more than just random features. The most widespread oscillation prevailed from 3,500 to 3,000 yr b.p. in north-western Sahara, Mauritania, Niger, Chad and the Kenyan–Ethiopian rift (Fig. 5). Runoff from Tibesti into Libya increased although no lakes developed. Since 3,000 yr b.p., desert conditions have once more descended on the central Sahara. Many East African lakes became dry or saline[2,13,14]. During the past 2 millennia, there have nevertheless been minor moister fluctuations of ~ 10^2 yr, affecting the rift valley, Lake Chad and possibly Mauritania[12,13,15,40–42] These show some coincidence in timing between 2,100–1,500 yr b.p., 1,200–1,000 yr b.p., and after AD 1,600.

Fig. 2 African lake levels for the glacial maximum 18,000 ± 3,000 yr b.p. Symbols as for Fig. 1.



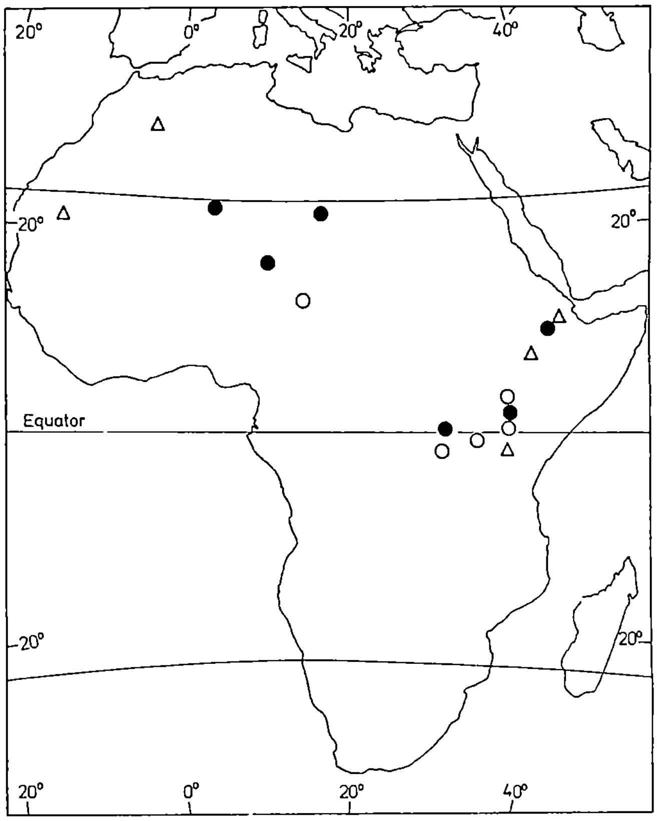


Fig. 3 African lake levels 11,000–12,000 yr b.p. Symbols as for Fig. 1.

Lake levels in the present century have oscillated quite widely on a time scale of 10–30 yr[12,41,43–45] though the fluctuations show less spatial coherence than in periods 1–3. Most lakes reached high levels between 1870 and 1895, rising again between 1950 and 1965 after several decades of low stand. The extent of Lake Chad peaked in 1965 and contracted dramatically during the Sahel drought[41].

Environmental implication

What are the implications of these profound changes in the abundance of surface moisture? We will focus on four time periods: (1) the glacial mode (21,000–12,500 yr b.p.), Fig. 2; (2) the late glacial (12,500–10,000 yr b.p.), Fig. 3; (3) the interglacial mode (10,000–5,000 yr b.p.), Fig. 4; and (4) the irregular decline from full interglacial conditions (5,000–0 yr b.p.), Figs 1, 5.

During period 1, desert and semi-desert conditions expanded over large areas of tropical Africa. Biogeographic evidence suggests that equatorial lowland forest was drastically reduced in extent and was restricted to refuges in West Africa (ref. 46, Hamilton, personal communication) and eastern Zaire. In the Sudan, desert conditions penetrated up to 450 km southwards into present semi-desert grassland, scrub and thorn savanna areas[47]. The net results were a widespread reduction in effective plant cover and substantial increases in albedo and in infrared emission (10–11 µm) from sand-covered areas.

There are limited indications of a reverse trend in northern and southern Africa. Mediterranean parkland and scrub may have intermittently invaded the steppelands flanking the Atlas[29]. South African data imply that montane grassveldt displaced semi-desert scrub in the Orange Free State before 17,000 yr b.p. and from 13,200 yr b.p. to 12,600 yr b.p. (refs 28, 36, 48). Events in between are uncertain.

Tropical African river systems responded in a complex way to decreasing plant cover and discharge[32,33,49]. Arguably,

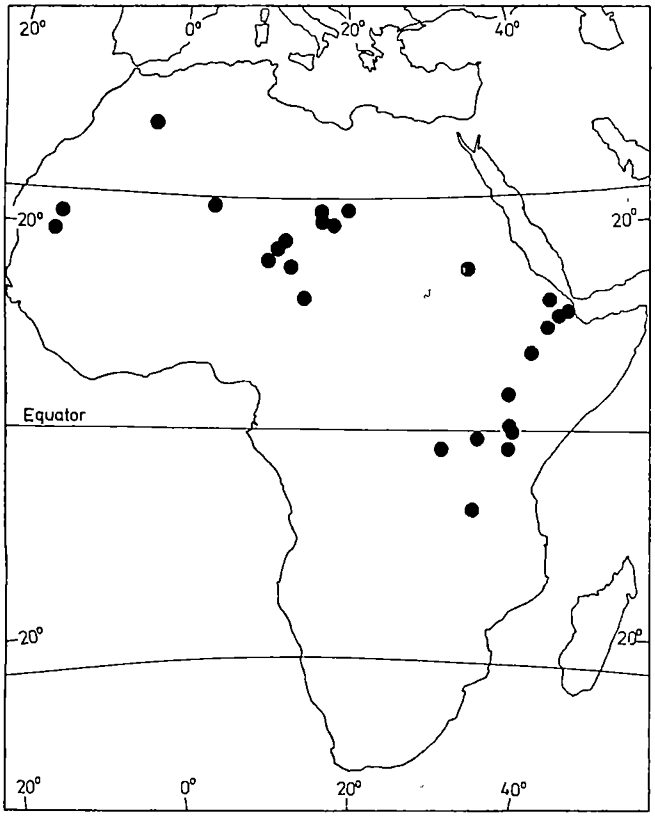


Fig. 4 African lake levels 8,000–9,000 yr b.p. Symbols as for Fig. 1.

the input of solutes to the oceans was greatly reduced, since many major rivers became highly seasonal or were blocked by dunes. The net change in clastic load is still in dispute[20,32], and was complicated by sea level lowering. But strong peaks of terrigenous dust in deep-sea cores suggest that aeolian transport of quartz and opalline silica to the Equatorial Atlantic culminated during glacial maxima[19,50–52].

The late glacial

Period 2 heralded a general restoration of forest vegetation across central Africa[46] which was accentuated after 10,000 yr. In the Sahara, semi-desert grassland, scrub and thorn savanna invaded huge expanses of former aeolian sands. Lowland forest species advanced 300–400 km north of their present limits in the Sudan[47]. Formation of lagoonal clays and swamp soils from 12,000 to 4,500 yr b.p. (refs 5, 33) has been of very great agricultural and economic importance.

The interglacial

We infer that during period 3, biological productivity and biomass greatly increased in tropical Africa, and that the surface albedo was substantially diminished by the expansion of open water, marshes and forest. Elephant, giraffe and antelope ranged comparatively unchecked over the Sahara[53], which must at times have existed only as relict desert areas isolated by corridors of gallery forest and swamp along major wadis[47,54]. Overflowing lakes established links between previously separate drainage systems[12,49]. The significance of these water connections for the spread of aquatic fauna and waterborne diseases is still insufficiently appreciated (see ref. 44). Traces of hippopotamus and crocodile are widespread even in the central Sahara[53]. Tropical freshwater molluscs, including the vectors of schistosomiasis, are preserved in Holocene deposits from as far north as Tibesti and Hoggar[55]. Malarial mosquitoes probably spread in the same way. The abundance of surface water had a significant role in the diffusion of Late Stone Age fishing cultures and later cattle herders across the huge region between Mauritania and Kenya[54,56]. Following this optimum, the decline of the plant cover and the retreat of the big game from the Sahara have been traced by Butzer[29] and Wickens[47].

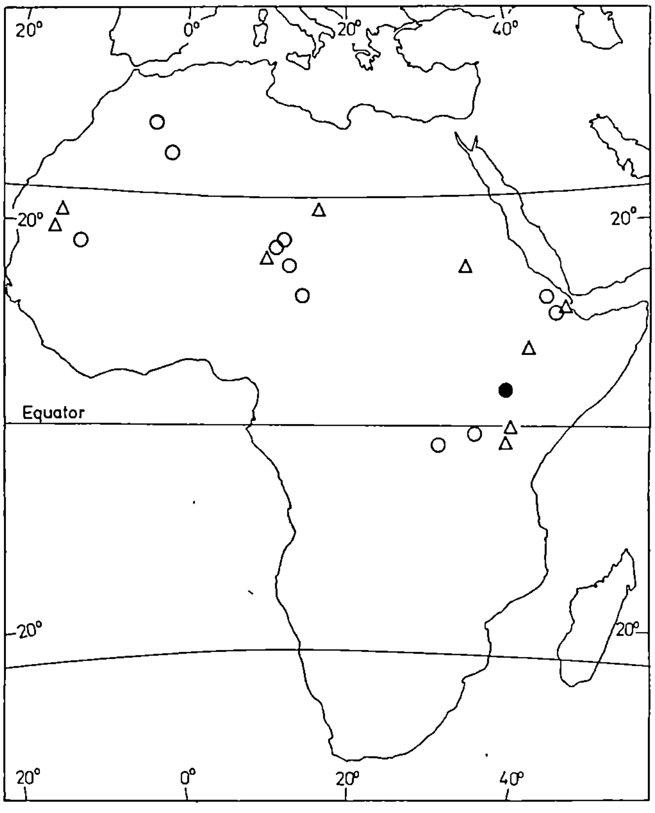
Climatic effects

Since the net radiative cooling of the atmosphere is counteracted mainly by liberation of latent heat[57], large low-latitude anomalies of precipitation and evaporation are of far reaching climatic significance. Past rainfall can be estimated from the extent of closed lakes, given realistic estimates of temperatures and runoff coefficients. It is assumed, for lack of data, that cloud cover and the seasonality of precipitation have remained constant. For period 1, maximum precipitation estimates for East Africa range from 54 to ~ 90% of that now, assuming a 3 °C temperature decrease[30,31,58]. For the Sahel, 15–20% is suggested by environmental indicators[47,59]. The high levels of mid-latitude lakes could be explained either by increased rainfall or by reduced evaporation caused by lower temperatures, but a 123% increase in rainfall has been computed for Alexandersfontein, based on a 6 °C fall in temperature[25]. Over Africa as a whole, the net effect is believed to have been a decrease in precipitation. Note, however, that no allowance has been made for the increased vigour of the trade wind circulation[61].

Rainfall estimates for period 3 range from 165% in East Africa (with temperatures equal to or above that of today)[2] to 200–400% or more in the Sudan and Mauritania[47,60]. The East African figures are probably the most representative of intertropical Africa for moisture transport computations.

Palaeoclimatic reconstructions for period 1 imply that (*a*) the meridional temperature gradient increased in both hemispheres but that the increase was largest in the Northern Hemisphere[61], (*b*) the vigour of the north-east trades was greatly enhanced[19,50–52], (*c*) Atlantic and Indian Ocean surface temperatures were up to 6–8 °C cooler in areas of upwelling[62–64], (*d*) the Atlantic oceanic polar front was displaced southwards

Fig. 5 African lake levels 3,000–4,000 yr b.p. Symbols as for Fig. 1.



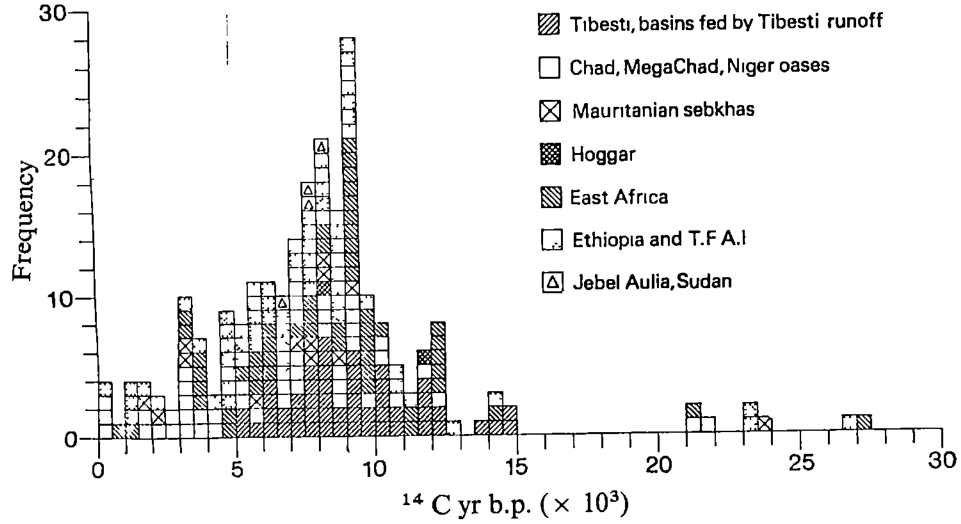


Fig. 6 Histogram of 238 ^{14}C dates from intertropical Africa which record high or intermediate lake levels. Lacustral phases before 21,000 yr b.p. are under-represented because of erosion and burial of their deposits.

to the latitude of Spain[62], (*e*) temperatures were 4–8 °C cooler over much of Africa[65], and (*f*) global atmospheric moisture transport was substantially reduced[66,67]. It has been inferred from (*a*) and (*d*) that the climatic belts contracted towards the meteorological Equator (*g*), and from (*a*) that the mean position of the Intertropical Convergence Zone (ITCZ) lay south of the geographical Equator (*h*)[68]. Figure 2, coupled with (*d*) and the evidence for very dry conditions in southern Europe[69–71], suggest that the Mediterranean winter rain belt was displaced south over northern Africa, promoting more frequent incursions of cold northerly air. It supports the view that the seasonal migration of the ITCZ and rainfall generation were much reduced, and is compatible with (*h*) although the evidence from southern Africa is barely sufficient to prove this point (refs 26, 27, 48; and A.T.G., unpublished).

Conclusions

We suggest that the widespread increase in rainfall which culminated between 8,000 and 9,000 yr b.p. was a direct response to increasing ocean temperatures. Figure 6 bears a pronounced similarity to the retreat pattern of polar water in the North Atlantic[72] including the abortive event seen in period 2. The onset of warming in the western Indian Ocean is dated at 10,000 yr b.p. (refs 64, 73). It is highly significant that this climax of latent heat transport over Africa coincided with the final stagnation and disappearance of the northern hemisphere icesheets[74,75] and with a major rise in sea level[76].

Figure 4 implies a significant intensification and greater seasonal displacement of the ITCZ. Data from south of Rukwa are still insufficient to determine whether its mean position lay further north than now[77]. The occurrence of high lake levels in the Holocene in Algeria and Israel[22,35] suggests a simultaneous deepening of the Mediterranean cyclonic rain belt, although the minimal extent of northern hemisphere ice ~ 5,000 yr ago might allow tropical disturbances to penetrate further north over the Sahara[78].

Historical lake-level rises seem to have been caused by regional increases in rainfall and runoff[43] but are broadly correlated with periods of decreased global temperatures[41,78,79] and are not a valid analogue for periods 2 and 3. The interplay of the circulations in the two hemispheres was rather different in the earlier periods. But individual years could possibly serve as analogues for periods 1 and 3 although the scale of the rainfall anomalies is smaller. We tentatively suggest the years 1971–72 in the Sahara as models for 21,000–17,000 yr b.p. and 1913 and 1970 for 17,000–13,000 yr b.p. Suitable analogues for period 3 appear to be 1952 and 1957 (refs 78–83). In 1951–52 the 100-mm isohyet was displaced 300 km north in Mauritania[78], but this advance had been reversed by 1972 (ref. 82).

We conclude that lake-level changes over Africa during the past 21,000 yr have reflected major shifts in precipitation regimes. The annual isohyet pattern has been displaced for periods of 10^2–10^3 yr to the extremes of its recorded distribution (see also ref. 84). These fluctuations have occurred on a sub-continental scale which is directly related to the global dynamics of the atmosphere and oceans. We thank W. W. Bishop, J. M. Bowler, G. L. Isaac, C. Vita-Finzi and M. A. J. Williams for comments. This work has been supported by the NERC, the Royal Society and the Department of Geography, Cambridge.

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Conformation of the lecithin polar group in charged vesicles

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The conformation of the polar headgroup of phosphatidylcholine in bilayer vesicles has been investigated using paramagnetic probes. The extended disposition of the polar group when bound to cations is maintained when anions are bound.

A BILAYER structure of phosphatidylethanolamine has been determined from X-ray diffraction studies on single crystals obtained from glacial acetic acid[1]. The structure shows the ethanolamine polar group to be approximately parallel to the plane of the bilayer, that is, perpendicular to the long chain fatty acid residues. Although some general structural studies of lecithin layers in water[2,3] suggest that its phosphorylcholine polar group lies parallel to the long chain residues, that is, perpendicular to the bilayer plane, other studies[4–6] suggest that the conformation is similar to that found in phosphatidylethanolamine. In all these studies the methods used for structure determination were relatively poor, often involving only one experimental parameter and uncertain theoretical simplifications. We report here a structural study of the polar group of lecithin bound by cations in vesicles exposed to aqueous solution using lanthanide shift and relaxation probes which give us some twelve parameters.

We used paramagnetic probe analysis[7], involving proton and phosphorus nuclear magnetic resonance (NMR) spectral perturbations of the high resolution spectra of sonicated aqueous lecithin dispersions. The experimental procedure was as follows.

Material and preparation of vesicles

Egg lecithin (grade I), egg lysolecithin (grade I) and glycerophosphorylcholine (as the $CdCl_2$ complex) were purchased from Lipid Products, South Nutfield. Dilauroyl lecithin was synthesised by acylation of L-α-glycerophosphorylcholine cadmium chloride using the appropriate acid chloride. The purity of the phospholipids was checked by thin-layer chromatography and that of glycerophosphorylcholine by paper chromatography as before[8]. Single-layer vesicles (~300 Å diameter) were prepared by ultrasonication of a dispersion (typically 1%) of egg lecithin or dilauroyl lecithin in D_2O (99.8%, Norsk Hydro)[8,9] under nitrogen.

Lanthanide chloride solutions were prepared in D_2O as before[10] to a final nominal *p*H of 5±0.2. Titrations were carried out by addition of aliquots of the required stock solution to the sonicated lecithin dispersion up to a lecithin to metal molar ratio of 5:1.

Stock solutions of $K_3[Fe(CN)_6]$ and $K_3[Cr(CN)_6]$ in D_2O were prepared after recrystallisation of the compounds (obtained from BDH and Alfa Inorganics, respectively). Aliquots of these solutions were added to the sonicated lecithin dispersion, in the case of $Fe(CN)_6{}^{3-}$ up to a lecithin to anion molar ratio of 10:1.

Table 1 Assignment of the ^1H resonances of sonicated phosphatidylcholine dispersions in D_2O

$-CH_3$ (terminal)	0.88±0.01
$(CH_2)_n$	1.27±0.015
$CH{=}CH$	5.30±0.015
$-CH_2-(C{=}C)_2$	2.82±0.02
$-CH_2-C{=}C$	2.02±0.02
CH_2-C-CO	1.57±0.02
CH_2-CO	2.38±0.02
CH_2OCO	4.41±0.03
CHOCO	5.32±0.02
CH_2OP	4.02±0.02
CH_2OP (choline)	4.28±0.01
CH_2N	3.69±0.01
$N(CH_3)_3$	3.25±0.01

Chemical shifts are expressed in p.p.m. downfield of TSS.

Table 2 Spin-lattice (T_1) relaxation times (s) of the proton resonances of egg lysolecithin and sonicated egg lecithin dispersions in D_2O

	Lysolecithin	Egg lecithin*
$-CH_3$(terminal)	0.62±0.03	0.52±0.03
$(CH_2)_n$(chains)	0.41±0.03	0.38±0.03
$CH=CH$		0.42±0.03
$-CH_2-(C=C)_2$		0.39±0.03
$-CH_2-C=C$		0.39±0.02
CH_2-C-CO	0.29±0.02	
CH_2-CO	0.34±0.02	0.31±0.02
CH_2OCO(glycerol)	0.38±0.04† 0.28±0.03	
CHOCO		
CH_2OP	0.40±0.02	
CH_2OP(choline)	0.35±0.02	0.32±0.02
CH_2N	0.40±0.03	0.31±0.02
$N^+(CH_3)_3$	0.36±0.02	0.32±0.02

*The values given are the average T_1 values for the internal and external groups of the lecithin bilayer as is evident from the determination of spin-lattice relaxation times in the presence of 0.01 M $EuCl_3$ when the internal and external polar group resonances were clearly separated.

†The two protons of the $C\underline{H}_2OCO$(glycerol) group are inequivalent and clearly resolved.

^1H spectra were recorded on a Bruker 270-MHz instrument operated in the Fourier transform mode and using a superconducting magnet (Oxford Instrument Co.). Trimethylsilylpropane sulphonate (TSS) was used as internal standard. Spin lattice (T_1) relaxation times were obtained by means of a 180°-t-90° pulse sequence.

^{31}P spectra were run on a Bruker WH90 spectrometer operating at 36.436 MHz and equipped with heteronuclear decoupling facilities. $(Me_3O)_3PO$ was used as internal standard. All spectra were recorded at an ambient temperature of 26 °C unless otherwise stated.

Determination of binding site in metal complex

A complete assignment of the proton high resolution NMR spectrum of lecithin was required initially. This was based on work reported in the literature together with spin decoupling and nuclear Overhauser experiments[11]. The confirmed assignments of the observed resonances from groups in the fatty acid chains and the lecithin polar group are given in Table 1. It is worth noting that the $-CH_2OP$ protons (P represents phosphate) of the glycerol are not equivalent, indicating that unlike the CH_2 groups of the choline which are equivalent, the glycerol part of the molecule has a rigid conformation. Studies of line widths and relaxation times have led to this conclusion previously[9,12]. Spin-lattice relaxation time (T_1) measurements carried out on lysolecithin clearly showed that the T_1 values are shortest in the glycerol part of the headgroup (Table 2).

Before using the paramagnetic lanthanide cations, we added diamagnetic La(III) ions to the lecithin vesicles in aqueous solution to facilitate separation of diamagnetic from paramagnetic effects. As La(III) cations had no perturbing influence on the NMR spectrum, we concluded that the observed shifts with the other rare earth ions were due to paramagnetic perturbations alone. As far as protons are concerned, the shifts due to a metal ion bound to a diester phosphate are known to originate totally from pseudocontact[13] terms. Two independent produres showed lanthanide ions to bind only to the phosphate.

(1) The observed ratios of the shifts of the different proton resonances (Table 3) were independent of shift probe[14,15]. The shift ratio for the ^{31}P resonance relative to the $POCH_2$ proton shifts was, however, strongly dependent on the lanthanide cation. This dependence must be due to contact perturbations (see later) through-bond from the metal.

(2) The measurement of line broadening effects and T_1 values in the presence of Gd(III) as a relaxation probe showed that the nearest protons to this metal were those of the CH_2OP (glycerol) and the $POCH_2$ (choline) groups, but that the phosphorous atom was considerable nearer than either of these (Table 3).

We conclude that binding of the metal ion to the lecithin molecule occurs at the phosphate group only. Table 3 also shows the relative broadening of various resonances by Gd(III) and this gives an indication of the distance of Gd(III) to the different protons of the polar group and fatty acid chains.

The experiments described above define neither the stoichiometry nor the conformation of the metal binding to the phosphate group. Of interest in the study of the binding of metal ions to a mono- or bilayer structure is the possibility that binding could occur to two centres simultaneously, or even that the observed pseudocontact shifts are a sum comprising contributions from one lanthanide ion bound to a given ligand and another bound on a neighbouring ligand. Two experimental procedures enabled us to examine these possible complications. First, the induced pseudocontact shifts were studied at different degrees of metal binding to the bilayer (that is, with increasing lanthanide concentration up to a lecithin–metal molar ratio of 1:5) and the derived shift ratios were shown to be independent of the number of lanthanide cations bound to the exposed surface of the vesicle in the concentration range used. Second, the shift ratios were determined for vesicles in which the lecithin was diluted by the introduction of an 'inert' molecule, monoolein, to the bilayer. We have found that the shift ratios of the lecithin protons at a given lanthanide cation concentration are independent of monoolein to lecithin molar ratio in the bilayer from zero to about 1.0. We conclude that the observed shift ratios are those for molecules bound to the lanthanide cation and are independent of next near neighbour effects. Third, the stoichiometry of Ln(III):phospholipid at saturation was determined using competition between Ln(III) binding to acetate and to the bilayer. This method showed that one Ln(III) binds to two phospholipid molecules and is therefore effectively chelated on the surface of the bilayer. Strong support for this conclusion was obtained from the shift data with various lanthanides.

As stated above, the shifts for all the proton resonances were measured in the presence of several lanthanide cations—Pr(III), Nd(III), Eu(III), Ho(III), Tm(III) and Yb(III). The magnitude of the shifts for the different ions was in the order predicted by Bleaney[16] and the shift ratios were independent of lanthanide cation even for the usually very anomalous cation, Tm(III)[17]. In the first place whatever the conformation of the headgroup of the lipid, this result shows that the mode of binding in the metal ion–polar group complex is very well defined and independent of cation size. Normally Tm(III) and closely related Ln(III) cations bind to an oxyanion in a different

Table 3 Ratios of the pseudocontact shifts and distances from the probe (r_{ij})† obtained for the ^1H and ^{31}P resonances of lecithin relative to the induced perturbation on the CH_2OP(choline) signal

	R_{ij}*	r_{ij}†
$N^+(CH_3)_3$	0.34±0.04	1.24±0.04
CH_2N	0.55±0.05	1.12±0.04
CH_2OP(choline)	1.00	1.00
^{31}P	3.10±0.40	0.65±0.09
CH_2OP(glycerol)	0.65±0.06	1.03±0.05
CHOCO	0.18±0.08	1.20±0.06
CH_2OCO	0.10±0.06	1.16±0.05
CH_2-CO		1.81±0.12
CH_2-C-CO		1.97±0.14

*Shift ratios, R_{ij}, were independent of the different lanthanide cation used: Pr(III), Nd(III), Eu(III), Ho(III), Tm(III) and Yb(III). The values given relate to studies on egg lecithin, egg lysolecithin and dilauroyl lecithin when the derived shift ratios were invariant within experimental error. No shifts of the protons of the hydrocarbon chain protons were detected in the conditions used.

†Relative relaxation data in the presence of Gd(III) were obtained both by spin-lattice relaxation time (T_1) measurements and line broadening effects (determined by difference spectroscopy) The ratios, r_{ij}, given are those of relative distances from the probe derived from the isotropic (r^{-6}) dependence of the induced relaxation[17].

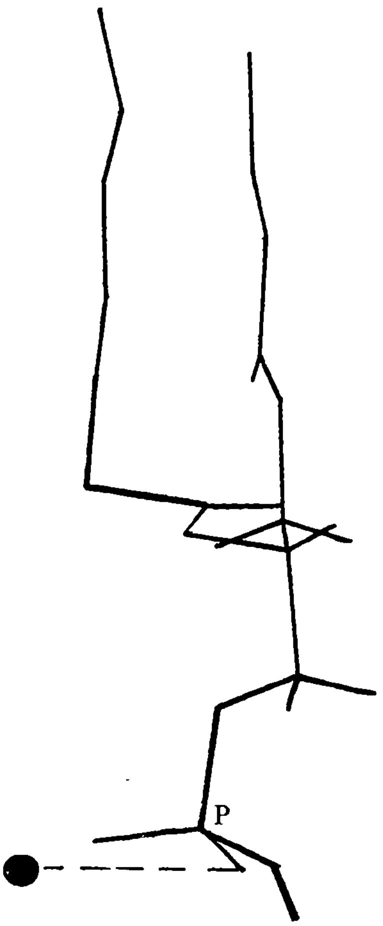

Fig. 1 Metal coordination scheme. The symmetry axis lies along the M–O bond.

manner from the other lanthanides[17]. The only previous circumstance in which Tm(III) has been seen to behave in the same way as the other lanthanides in aqueous solution is in binding to ligands which have a very constrained coordination geometry, for example, bidentate (chelate) ligands[18]. This observation confirms the deduction from the binding studies that the lecithin–metal complex is a surface 2:1 complex. The constrained conformation is independent of the size of the lanthanide cation, and as we show below, is also maintained when anions are bound. Before turning to anion binding, however, we wish to analyse the conformation of the polar group in detail, using the lanthanide shift probes.

Conformational analysis

The proton shift ratios in the presence of the different Ln(III) cations were found to be independent of the lanthanide cation chosen, and we are therefore justified in the assumption that the site of binding has effective axial symmetry, and that the shifts on the protons are pseudocontact in origin[13]. As shown above, however, the shifts on the ^{31}P resonance for the different lanthanides contain both a contact and a pseudocontact term. These were separated by the methods used previously[10]. The pseudocontact shift ratio on the phosphorus so obtained (Table 3) merits comment. In comparison with the shift ratios $^{31}P:POCH_2$ in other phosphate esters, this value is small[10]. This is a clear indication that the metal is not bound to the phosphate of lecithin in the same way as was seen for 5′-AMP, for example[7,13], when the metal was coordinated to two of the free phosphate oxygens. This is consistent with two observations. First, extrapolation of the measured ^{31}P shifts to infinite lanthanide concentration, using a conventional formula for the dependence of binding to the bilayer upon concentration, gave a value lower than the fully bound shift in simple phosphate ester–lanthanide complexes. Second, the variation of this shift from lanthanide ion to lanthanide ion is not the same as that found in other phosphate esters[10]. The magnitude of the ^{31}P contact shift due to the lanthanides is also different for the lecithin phosphodiester group from that for all other phosphate esters studied by us[7,10,13].

Further demonstration of the degree of difference in the binding mode between lecithin and small model phosphate diesters was obtained from a comparative study of the shifts induced on the protons of glycerophosphorylcholine by lanthanide ions. (Glycerophosphorylcholine has the same chemical formula as the polar group of lecithin.) The shift ratios and the absolute shifts were quite different from those of lecithin and will form the subject of a separate communication.

In the light of these facts a conformational search for the metal ion–lecithin complex could not be based on previous coordination geometries for phosphate ester binding to lanthanides. The results demand an initial computer search for the metal position with respect to the phosphate binding site and the solutions generated must be consistent with the metal ion being in contact with at least one oxygen atom of each of two lecithin phosphate groups, as shown by the ^{31}P contact shifts. To proceed with such a metal position search we require a rigid frame portion of the molecule[17], possessing observable nuclear resonances whose spectral perturbations can be used to define the metal position in space. We decided to use the protons of the glycerol group bound to the fatty acid chains since these protons are known to be highly immobilised (see above). Their relative geometry was taken from the structure of phosphatidylethanolamine[1]. The diamagnetic spectrum of phosphatidylethanolamine gives a similar proton NMR spectrum as that of lecithin but for the absence of the trimethylammonium resonance. The computational search allowed free rotation about the $-CH_2-O-P$ bonds of the phosphoglycerol unit but spatial overlapping of atoms was forbidden[7].

The search was made using only one lecithin molecule per metal ion, although there are two lecithin molecules bound per cation. We have shown that the metal complex has axial symmetry. In such circumstances a symmetrical chelating group XA.AX, where A is the binding group (here phosphate), must bind in such a way that groups X and A are related by a rotation about the axis of symmetry or by a centre of inversion at the metal. Thus the metal ion position search can be based on half of the chelate, XA. The solutions are then open to inspection in terms of a constructed dimer which must of course form part of a close-packed bilayer film.

The procedure gave the metal position of Fig. 1 which is consistent with the broadening and T_1 data using Gd(III) as relaxation probe. The position of the metal is not that previously found for the lanthanide complexes of phosphodiesters. The conformation of the choline group was then studied using the determined geometry of the metal fatty acid glycerophosphate unit. Free rotation was permitted about the bonds $P-O-CH_2-CH_2-N$ and the conformation giving the best fit to both shift and relaxation data was determined. The centroid of the only family of closely related conformations to fit the experimental data is shown in Fig. 2.

This conformation has the choline approximately perpendicular to the bilayer plane, quite different from the structure found for phosphatidylethanolamine in acetic acid[1]. The conformation is also very different from that given by Barsukov *et al*[19]. Relevant internal dimensions and the various torsion angles of the extended group are given in Fig. 2. The study of Yeagle *et al.*[4] is compatible with this structure although their interpretation is different, and the ^{13}C NMR study[20] gives data which, for carbon atoms that do not suffer contact shifts, are in agreement with the conformation.

Returning to the 1:2 stoichiometry, we must now place the second lecithin molecule in relation to the first, this position being strictly defined by the rotation about the symmetry axis or centre of inversion (see above).

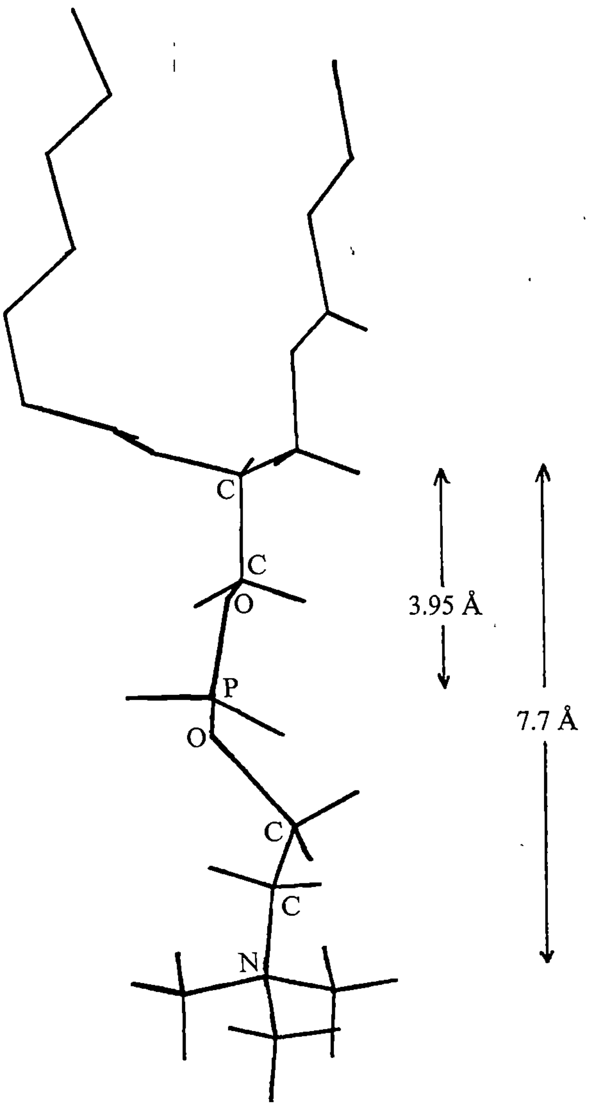

Fig. 2 Conformation of the lecithin polar group. This view is related to Fig. 1 by a rotation of −40° about a vertical axis. The N–C–C–O torsion angle (α_5) is $161\pm16°$ while that about the P–O–C–C bond (α_4) is $207\pm7°$. The phosphodiester group is in a *gauche-trans* conformation ($\alpha_3=201\pm6°$, $\alpha_2=3\pm6°$, $\alpha_1=190\pm5$). Torsion angles are defined as in ref. 1.

The axis of symmetry passes along the M–O bond (O of phosphate) so that only one position is permitted for the second molecule which is in the opposite positive quadrant of the shift cone. The resulting bilayer chelate disposes the lecithin molecules about the metal so that they are in the opposite positive regions of the bimodal shift cone function. The fatty acid chains of the lecithin molecules remain in contact with one another and still form a simple bilayer film.

It is interesting to note some internal distances of this chelate formed from the two lecithin molecules. The M–O distance is 2.7 ± 0.1 Å and the separation between the two planes through each N atom (and perpendicular to the bilayer plane) is 11.8 Å.

Paramagnetic anion probes

The conformation of Fig. 2 is open to further experimental investigations through studies of anion binding using probes such as $\mathrm{Cr(CN)_6}^{3-}$ (relaxation) and $\mathrm{Fe(CN)_6}^{3-}$ (shift). Addition of the first of these anions to the aqueous solution of lecithin bilayer vesicles affected the lecithin ^{1}H NMR spectrum considerably. The order in which resonances were broadened was easily followed by difference spectroscopy and was

$\mathrm{N^+(CH_3)_3}$ > $\mathrm{N{-}CH_2}$ > $\mathrm{H_2C{-}O}$ (choline) > $\mathrm{O{-}CH_2}$ (glycerol)

The shift ratios with $[\mathrm{Fe(CN)_6}]^{3-}$ were found to be

$\mathrm{(CH_3)_3N^+.CH_2.CH_2.OP}$
1.00 0.9 0.5

There was no measurable shift or broadening of other resonances. The data show that the lecithin polar group binds anions in such a manner that the $[\mathrm{Cr(CN)_6}]^{3-}$ is distant from the P–O–$\mathrm{CH_2}$ glycerol group of the lecithin. It is difficult to envisage any conformation of the polar group of lecithin which does not have the $\mathrm{N^+(CH_3)_3}$ group extended away from the bilayer (as in Fig. 2) but could still give this result. Thus we picture the anions binding considerably further from the fatty acid chains than the cations. This result can be confirmed directly by a consideration of the relative broadening of the protons of the chains induced separately by Gd(III) and $\mathrm{Cr(CN)_6}^{3-}$. It has been found that while Gd(III) causes measurable broadening of the chain protons, no such effects are observed in the presence of $[\mathrm{Cr(CN)_6}]^{3-}$. The final conformation is shown in Fig. 2.

Experiments using lysolecithin permit a detailed confirmation of the above conformation. The proton NMR spectrum of lysolecithin is sufficiently well resolved that we can follow not only the spectral shifts and relaxation times when paramagnetic ions are added, but we can also determine and analyse the coupling constants. Our data show that the conformations of the head groups of lysolecithin and lecithin are the same using both paramagnetic cations and anions to determine distances by relaxation methods and using paramagnetic cations to determine vectors by shift probe methods. As above, data were collected for both protons and phosphorus. Analysis of coupling constants for the choline head-group of lysolecithin gave data very similar to those for choline itself, indicating that the headgroup is in the *trans*[1] configuration. This is the configuration shown in Fig. 2, deduced from shift and relaxation data. The coupling constants are affected only slightly by the addition of metal ions, or increase in temperature.

We do not wish to overstress the significance of the conformation shown in Fig. 2. Our methods[7,17] give the central solution of a family of conformations all of which, individually and therefore collectively, will fit our data. A considerable population of conformations much outside this family cannot be present, however, for the family is defined by twelve parameters from three methods with different vector functions. Here the family of solutions is relatively small. Finally we cannot exclude motion within the family or to some degree outside it.

Phospholipid polar group conformations

The greatest interest surrounds the disposition of the lecithin polar group (choline) for it is so different from that of ethanolamine. If we consider the two groups, $\mathrm{-N(CH_3)_3^+}$ and $\mathrm{-N^+H_3}$, then while the first binds most readily to anions such as $\mathrm{RSO_4^-}$, $\mathrm{ClO_4^-}$ and anions high in the chaotropic series, the second polar group binds most readily through hydrogen bonds, that is to $\mathrm{RCO_2^-}$ and $\mathrm{ROPO_3^{2-}}$. Sterically $\mathrm{-N^+H_3}$ is much smaller than $\mathrm{-N(CH_3)_3^+}$. Presumably together these two factors generate the observed difference in stereochemistry of the two polar groups in their lipid bilayers. We can extend this finding to a consideration of the binding of other molecules to the bilayer surface. Thus phospholipid–protein interactions may show high selectivity. Using the lanthanide probes, it should be possible to map out the position of added molecules lying in the membrane.

The different polar group conformations in the two types of phospholipid may well also give rise to their different behaviour on a macroscopic level. The extended orientation of the phosphatidylcholine polar group could result in a dipolar repulsion between bilayers and the small rate of bilayer fusion[21]. This same property of lecithin may also be the cause of the asymmetric distribution of phospholipids between the inner and outer layer of biological membranes. In erythrocyte membranes, for example, those phospholipids incorporating the $-N^+(CH_3)_3$ group have been found to be on the outer membrane surface while phosphatidylethanolamines and phosphatidylserines are on the inner membrane surface[22]. The accumulation of lecithin on the external membrane surface might also inhibit membrane fusion and thus contribute to the stability of cell membranes. The conformational differences between phosphatidylcholine and phosphatidylethanolamine which this work exposes may well account for the differences in their hydration properties[21].

This work is supported by the MRC.

Note added in proof: The assignment of the proton high resolution NMR spectrum of phosphatidylethanolamine bilayer vesicles has now been completed. Both $-POCH_2$ and $-CH_2N$ appear upfield of their chemical shift positions in the spectrum of lecithin consistent with a different polar group conformation in the two types of phospholipid.

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letters to nature

The interplanetary magnetic field structure

THE interplanetary magnetic field is the extension, by the solar wind, of the solar magnetic field[1]. The detailed connection between the solar and interplanetary fields depends, however, on the precise manner in which the wind originates in the solar atmosphere and involves a number of complicated dynamical questions which are not yet fully understood[2]. Thus knowledge of the three-dimensional structure of the interplanetary magnetic field is important to our understanding of the solar atmosphere and solar wind flow. Up to now, direct measurements have been confined to the close vicinity of the solar equatorial plane; as a result our ideas about the overall structure of the interplanetary field are speculative. Here we suggest that the cosmic-ray flux provides some indirect information about the morphology of the interplanetary field.

It is well known that the interplanetary magnetic field divides into two or four clearly-defined sectors[3], the field in each sector having a constant average sense, towards or away from the Sun. If the fine-scale structure of the photospheric field is ignored, the average field shows large unipolar regions extending over the surface of the Sun. The boundaries between these unipolar regions run in a nearly N–S direction, extending to ~ 40° in latitude[4,5]. The positions of these boundaries, at the solar equator, correlate with the positions of the sector boundaries of the interplanetary field observed at the orbit of the Earth[6]. For this reason it is generally presumed that the sectors of the interplanetary magnetic field correspond to the large solar unipolar regions and persist to relatively high latitudes[7], and that the sector boundaries have an orientation nearly perpendicular to the solar equatorial plane.

This conventional view of the interplanetary magnetic field's three-dimensional geometry seems to disagree with measurements showing a seasonal variation in the sector structure[8-12]. During the six months when the Earth is above the solar equatorial plane those sectors in which the field has the same sense as the north polar magnetic field of the Sun are broader than those sectors in which the field corresponds to the south polar field; and conversely during the six months of the year when the Earth is below the solar equatorial plane. The amplitude of the seasonal bias is large (~ 68%) and in addition, its phase reverses at the same time that the overall polar magnetic field of the Sun changes sign.

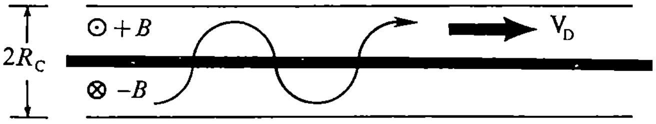

Fig. 1 All positively charged high energy particles, whose centres of gyration fall within one cyclotron radius, R_c, of the surface separating north polar and south polar fields in the solar magnetic cavity, migrate with an average velocity $\langle V_D \rangle$ in the direction of $\nabla \times B$.

These observations of the interplanetary magnetic field's sector structure seem to suggest that the field in each hemisphere of the solar cavity (north and south) has the same polarity as the average magnetic field at the corresponding solar pole, and that the surface, near the equatorial plane, which separates the two polarity regions is only slightly warped[2,13,14]. In this picture the observed sector structure results from corotation of the warped surface past the Earth with the angular velocity of the Sun.

Assume for simplicity that, at the orbit of the Earth, the warped surface has the shape of a sinusoid

$$z = z_0 \cos\varphi \qquad (1)$$

where z is the distance of the surface from the solar equatorial plane and φ is solar longitude. The observed 68% amplitude of the seasonal polarity bias implies that $z_0 \simeq 0.25$ AU. Thus, on the basis of this unconventional picture of the interplanetary magnetic field one would not expect the sectored structure to persist beyond ~ 15 ° of solar colatitude. (By way of contrast solar wind velocity stream structures are known to extend to high latitudes[15].)

Forbush pointed out that the anisotropy which gives rise to the total observed diurnal variation of the high energy cosmic-ray flux is not constant. The diurnal variation apparently consists of two components[16-19]. The dominant component is constant and corresponds to the corotational anisotropy of cosmic rays. A smaller component, ~ 25% of the total diurnal variation, corresponds to an anisotropy vector which is aligned approximately with the interplanetary magnetic field and which oscillates about a zero mean with a full period equal to the 22-yr oscillation time of the solar magnetic field.

We have shown elsewhere[13,14] that the unconventional picture of the interplanetary magnetic field described above leads in a natural way to the, heretofore unexplained, varying component of the diurnal anisotropy. Briefly, all high energy particles whose centres of gyration fall within one cyclotron radius of the discontinuity separating north and south hemispherical fields undergo a rapid drift $\langle \mathbf{V}_D \rangle$ in the direction of $\nabla \times \mathbf{B}$, as illustrated in Fig. 1. Since solar modulation produces a radial gradient of cosmic rays, $\mathrm{d}U/\mathrm{d}r > 0$, the drift gives rise to an effective source, S, of cosmic rays

$$S = -\langle \mathbf{V}_D \rangle_r \frac{\mathrm{d}U}{\mathrm{d}r}$$

where $\langle \mathbf{V}_D \rangle_r$ is the radial component of the average drift. $\langle \mathbf{V}_D \rangle$ changes sign from one eleven-year half period of the cycle to the next. S is superimposed on the usual convection–diffusion distribution of cosmic rays. Diffusive reduction of S gives rise to the observed solar-cycle variation in the diurnal anisotropy of high energy cosmic radiation. We know of no other way to account for variation with a 22-yr period[20]. (Quenby and Hashim[20] have discussed the variations in the diurnal anisotropy but gave no natural explanation of the 22-yr cycle.)

Altogether the simple model for the interplanetary magnetic field that we have outlined above provides natural and elementary explanations for two independent and unrelated observational facts. The conventionally accepted field morphology does not lead in any obvious way to either the seasonal bias in the sector structure or to the solar magnetic cycle periodicity of the diurnal variation of energetic cosmic rays.

Since magnetic field lines trace the solar wind flow, comparison of the interplanetary field configuration with the structure of the solar field should provide a valuable constraint on realistic models of the origin of the solar wind. In this regard the discrepancy between the large latitudinal extent of the photospheric sector structure and the apparently small extent of the interplanetary sector structure raises an interesting question. Several resolutions of the discrepancy suggest themselves. For example, the solar wind may originate high in the solar atmosphere where magnetic stresses have simplified the field's geometry. Or, alternatively, it is thought that coronal holes may produce a large fraction of the solar wind. Since large coronal holes are generally present in the polar regions of the Sun, it is possible that most of the solar wind comes from high latitudes and that the latitudinal extent of the sectors is depressed as the polar flow fans out to fill the sphere. A high latitude origin for the solar wind has previously been suggested to account for features of the 11-yr modulation of cosmic rays[21]. (Remote measurements of the solar wind velocity through radio scintillations[22] suggest that any fanning out of the solar wind would have to occur $\lesssim$ 10 or 15 solar radii from the Sun.) Either of these two possible resolutions of the sector structure discrepancy is consistent with the observation that no more than ~ 10% of the total photospheric magnetic flux appears in the interplanetary field[6].

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The microwave spectrum of HNC: identification of U90.7

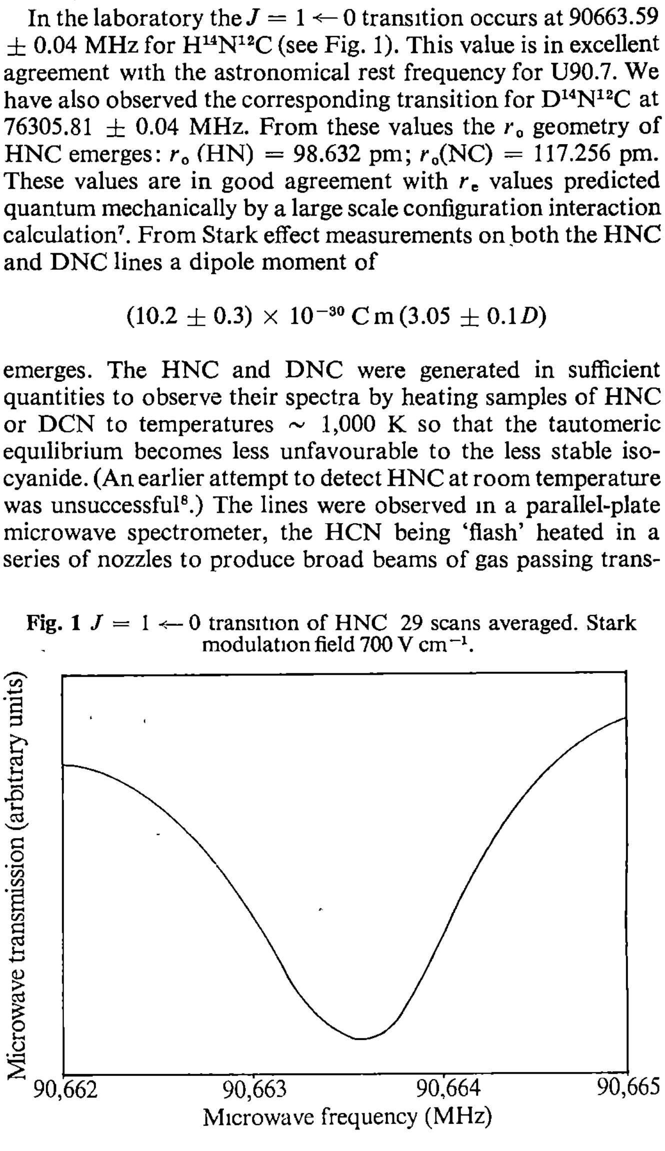
THE detection and characterisation of hydrogen isocyanide, HNC, was for a long time a challenge to science, the first real progress coming from matrix-isolation studies of its infrared spectrum in 1963[1] and it has more recently been detected by its infrared chemiluminescence in certain flames involving atomic nitrogen[2]. In 1971 an interstellar line (U90.7) was attributed by radioastronomers[3,4] to this molecule, the best estimate of the rest frequency of the line being[6] 90663.59 ± 0.15 MHz. The possibility that this line represents the $J = 2 \rightarrow 1$ transition of Si C has also been advanced[5]. We have now succeeded in generating HNC in the gas phase and observing its microwave spectrum. The agreement between our observations of the frequency of the $J = 1 \leftarrow 0$ transition and the radioastronomical data render the identification of U90.7 as HNC secure.

In the laboratory the $J = 1 \leftarrow 0$ transition occurs at 90663.59 ± 0.04 MHz for $\mathrm{H^{14}N^{12}C}$ (see Fig. 1). This value is in excellent agreement with the astronomical rest frequency for U90.7. We have also observed the corresponding transition for $\mathrm{D^{14}N^{12}C}$ at 76305.81 ± 0.04 MHz. From these values the r_0 geometry of HNC emerges: r_0 (HN) = 98.632 pm; r_0(NC) = 117.256 pm. These values are in good agreement with r_e values predicted quantum mechanically by a large scale configuration interaction calculation[7]. From Stark effect measurements on both the HNC and DNC lines a dipole moment of

$$(10.2 \pm 0.3) \times 10^{-30}\ \mathrm{C\,m}\ (3.05 \pm 0.1 D)$$

emerges. The HNC and DNC were generated in sufficient quantities to observe their spectra by heating samples of HNC or DCN to temperatures ~ 1,000 K so that the tautomeric equilibrium becomes less unfavourable to the less stable isocyanide. (An earlier attempt to detect HNC at room temperature was unsuccessful[8].) The lines were observed in a parallel-plate microwave spectrometer, the HCN being 'flash' heated in a series of nozzles to produce broad beams of gas passing trans-

Fig. 1 $J = 1 \leftarrow 0$ transition of HNC 29 scans averaged. Stark modulation field 700 V cm^{-1}.



versely between the plates and then condensed on a metal plate cooled with liquid nitrogen. Phase-locked klystrons (OKI 80V10A, OKI 90V10A) were used as sources and 33-kHz Stark modulation was used for detection of the signals.

In view of the manner in which we have generated HNC the possibility that the reported transition may be some rovibrational transition of thermally excited HCN must be considered. We eliminate this on the following grounds:

(1) The observed line is narrower than HCN lines. In our conditions the latter are observed as resolved triplets covering ~ 4 MHz, each component having a f.w.h.m. of ~ 800 kHz. The lines here reported have FWHM of 1,300 kHz, their unsymmetrical shape (Fig. 1) suggesting the possibility of hyperfine structure. The observed shape can be quite well simulated by computing the envelope of a multiplet of lines, each of FWHM 800 kHz, with the pattern corresponding to a quadrupole coupling constant $\chi \simeq 1.9 \pm 0.3$ MHz. A coupling constant of this magnitude is rather larger than that of other isonitriles (see ref. 9) and is larger than the theoretically predicted constant for HNC ($\chi = 0.9 \pm 0.5$ MHz)[7]. The value is also larger than the upper limit of $\chi \leqq 0.83$ MHz that Morris *et al.*[6] imply from their astronomical observations, and clearly, a higher resolution laboratory spectrum will be needed to obtain a measurement that is free from assumptions concerning either the line shape or the relative intensities of the hyperfine components.

(2) In addition to evidence against this possibility from the line width, the only feasible alternatives for HCN rovibrational transitions in this frequency range have been observed to fall at different frequencies (for example, the $J = 20$ (01^10) l-doublet, observed by Maki and Lide[10] at 93,813.85 MHz) or their frequencies, computed from the formulae presented by Maki and Lide[10], are well removed from those of the lines we report here (the l-doublets for 02^20 are at 75.3 GHz and 94.5 GHz for $J = 29$ and 31; for 03^10 they are at 72.0, 83.9 and 96.8 GHz for $J = 12, 13, 14$; for 03^30 they are at 72.9 and 93.9 GHz for $J = 39$ and 41). From our observations of the 02^00, $J = 1 \leftarrow 0$ transition at 89,088 MHz, it is clear that lines derived from more highly excited HCN would in fact have intensities well below our limit of detection in the present apparatus.

The agreement in frequency of the HNC line and that of U90.7 makes the identification of the latter now beyond reasonable doubt. Additional confirmation will come from detection of interstellar DNC at 76.3 GHz and of $HN^{13}C$.

We have also observed a weak signal (of the expected strength corresponding to the natural abundance of ^{13}C) from the $J = 1 \leftarrow 0$ absorption of $HN^{13}C$ at 87,090.6 ± 0.2 MHz, the identification being supported by the facts that the line has a similar shape to that of the main line and that the Stark effect is the same as that displayed by the main line. (We have learned that Dr R. Claude Woods and colleagues at the University of Wisconsin, using a somewhat different laboratory technique, have also observed the main line of HNC at 90,664 MHz.)

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Short period fluctuations of the equatorial electrojet

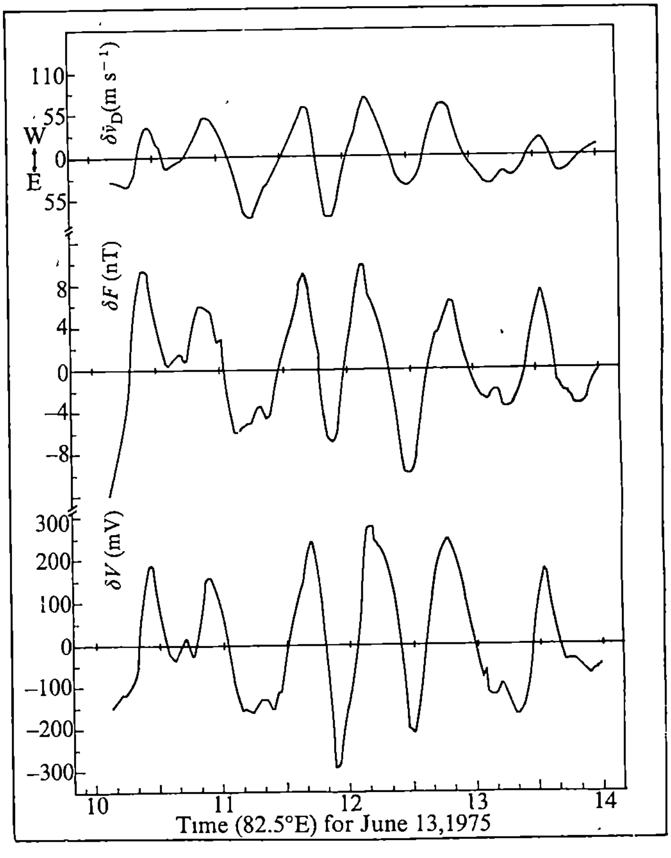
THE signal strength and Doppler frequency spectra of v.h.f. backscatter radar echoes from the equatorial electrojet are being recorded at Thumba near Trivandrum (dip ~1 °S). The v.h.f. radar operates on 54.95 MHz, with identical arrays of Yagi antennae for transmission and reception; the beam axis is orientated either vertically or obliquely at 30° to the vertical. Analysis of the radar backscatter signals and the magnetograms at Thumba has revealed that quasi-periodic fluctuations of the electrojet on time scales of a few tens of minutes are very frequent. We report here some typical experimental results on this phenomenon.

Radar echoes from only type II irregularities (gradient instabilities) with their characteristically broad Doppler frequency spectra have been analysed. For this type of a broad spectrum, a mean Doppler frequency $\bar{f}_D$ has been defined as

$$\bar{f}_D = \frac{\int_{-\infty}^{\infty} f_D \, P(f_D) \mathrm{d} f_D}{\int_{-\infty}^{\infty} P(f_D) \mathrm{d} f_D}$$

where $P(f_D)$ is the backscattered power per unit frequency at the Doppler frequency f_D. In practice, a summation is used over the usual range of f_D values (−175 Hz to +175 Hz). Thus $\bar{f}_D$ is a weighted mean (nearly the same but not identical to the

Fig. 1 Short period fluctuations of the backscatter signal strength (V), the geomagnetic field (F) and the E–W mean drift speed ($\bar{V}_D$) of electrojet irregularities, for June 13, 1975 ($A_p = 21$).



median value) and it shows systematic variations similar to those of the signal strength (and the magnetic field) whereas the Doppler frequency at which the power is maximum shows erratic variations quite often. If we make the usual assumption that the mean drift velocity of type II irregularities is horizontal in the east–west direction, then $\bar{f}_D$ is related to the mean east–west drift speed ($\bar{V}_D$) of the irregularities in the radar-illuminated volume by

$$\bar{f}_D = -(2 \cdot f_0 \, \bar{V}_D \cos\theta)/c$$

where f_0 is the radar frequency, c is the velocity of light *in vacuo* and θ is the angle of the radar beam axis with respect to the horizontal.

The slow daily variation or 'trend' has been removed in each case by subtracting a running mean from the observed values. A 60-min window is used for obtaining the running mean of the measured parameters which are available at 1-min intervals, except for the Doppler spectra which are recorded at 5-min intervals on most occasions; the 1-min values are then obtained by interpolation. The differences of the 1-min values from the running mean are further smoothed by taking a 6-min running average. The resulting values at 1-min intervals are the smoothed deviations from the mean, representing the fluctuations over time scales of tens of minutes. This procedure is found to bring out quite well the quasi-periodic variations in such a way that they are comparatively free from the longer period 'trend' as well as from the noise-like fluctuations of shorter time scales. (This procedure, however, needs a critical assessment when a quantitive spectral analysis of the quasi-periodic variations is to be made).

Figure 1 shows the quasi-periodic fluctuations in the mean values of (1) the backscatter signal strength (in terms of the receiver detector output voltage), (2) the strength of the total magnetic field (as measured by a Proton Precession Magnetometer), and (3) the mean east–west speed of the type II irregularities as deduced from the mean Doppler frequency of the backscatter signal. The remarkable similarity of the fluctuations in all three parameters and the persistence over a period of 4 h are the two typical features of such fluctuations observed on many days. On this particular day (June 13, 1975), one dominant period of ~ 25 min is present most of the time, but is suppressed part of the time by the interference of another perturbation with a similar period, or a perturbation of a different magnitude and period is dominant for some time. The largest amplitudes of the fluctuations on this day (which was a moderately disturbed day magnetically) are observed around noon and they constitute ~ 36% of the mean value for the signal strength, ~ 18% of the daily variation range of the magnetic field, and ~ 21% of the mean velocity of irregularities. In this figure as well as in the next, the uncertainty in the $\delta\bar{V}_D$ values is variable as it depends on the shape of the spectrum and the power levels measured; for the two cases presented here, the error bars are typically $\pm$ 10 m s^{-1}. On the other hand, the error bars on δV and δF consist of the measurement errors directly and are $\pm$ 10 mV for δV and $\pm$ 0.7 nT for δF.

On July 18, 1975, a magnetically quiet day, fluctuations of comparable amplitudes have been observed, as shown in Fig. 2. In this case, the largest amplitudes of the quasiperiodic fluctuations occur near 0800; the peak amplitudes are: 30% of the mean value for the signal strength, 21% of the daily variation range of magnetic field, and 36% of the mean velocity of the irregularities. No single dominant period persists over the interval of ~ 4.5 h; the perturbations of 20–30 min period are present in varying strength.

The main experimental result emerging out of this study is that the electrojet current (and not merely the strength and speed of the electrojet irregularities) undergoes large quasi-periodic fluctuations on many days, including magnetically quiet days. The fluctuations can arise from perturbations (1) in the electron drift speed relative to ions which are not perturbed or (2) in the ion drift speed relative to the electrons which remain unperturbed or (3) in both ion and electron drift speeds in such a way that the difference of electron and ion drift speeds undergoes fluctuations.

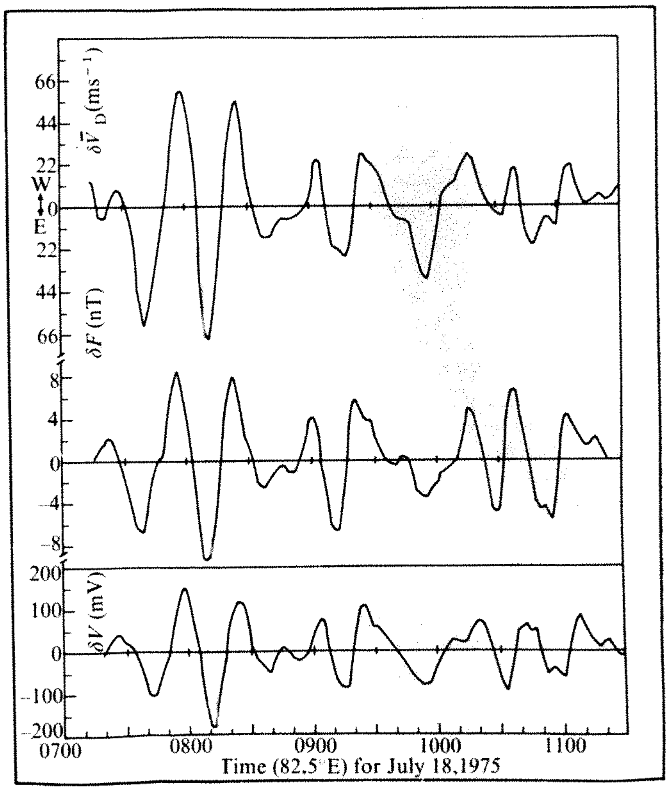


Fig. 2 As in Fig. 1, for July 18, 1975 (A_p = 6).

Theoretical treatments[1,2] of the motion of ionisation irregularities show that weak irregularities move with the velocity of electrons rather than that of ions. The type II irregularities we have observed are such weak irregularities and the variations in the drift speed $\bar{V}_D$, as observed with the v.h.f. radar, can be interpreted as variations in the speed of the electron drift. An interpretation in terms of variations in the speed of ion drift alone has both observational and theoretical objections: observationally, the measured fluctuations of $\bar{V}_D$ require unrealistically large winds of short periods; theoretically, wind-generated ion motion will give rise to a vertical polarisation field in such a way that the electrons and ions are constrained to move with the same horizontal drift speed[3]. The physical processes leading to perturbations (1) and (3) above can be understood only through a detailed quantitative theoretical study of the role of mesoscale winds (of gravity-wave origin) in the electrojet region. Such a study is in progress.

In the theoretical treatments[4-8] of gradient instabilities, the growth time for the type II instabilities is shown to decrease with increasing electron drift speed, but the dependence of the strength of the irregularities on the speed of the electron drift is not clear. The observations do show, however, that the strength of irregularities depends quite sensitively on the electron drift speed (see Figs 1 and 2).

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e.l.f. Hiss modulation at harmonics of the helium gyrofrequency

MANY plasma-wave phenomena involving the proton gyrofrequency have been observed in the ionosphere and magnetosphere but very few involving the helium gyrofrequency. One such is the phenomenon known as helium whistlers which were discovered by Barrington, Belrose and Mather[1] with the Canadian Alouette II satellite. Horita and Friesen[2] have also reported fine structure at harmonics of the proton and helium gyrofrequency on whistlers detected by the v.l.f. receiver on board the Canadian Isis II satellite. Another paper[3], reporting proton gyrofrequency phenomena on auroral hiss, mentioned parenthetically that extra low frequency hiss modulated at harmonics of the helium gyrofrequency has been observed. Here I give further details of the e.l.f. hiss modulation and discuss the region where the modulation may possibly have occurred.

Figure 1 shows the v.l.f. spectrogram made at the Quito station from the Canadian Isis II satellite on April 6, 1971 at 2329.00 to 2329.15 UT; at 2329.10 UT the satellite was at 8.5°S, 74.1°W, and at an altitude of 1,379 km. The superimposed horizontal lines indicate the harmonics of the helium gyrofrequency $f_{He} = 112.5$ Hz (which corresponds to an observed proton gyrofrequency $f_p = 4 f_{He} = 450$ Hz). This value was found by examining the spacing of the e.l.f. hiss modulation and is greater than the value of 60.7 Hz for the local helium gyrofrequency in the immediate vicinity of the satellite, suggesting that the source region is at a location or locations beneath the satellite. Note that the horizontal lines generally lie in the gaps of the e.l.f. hiss modulation for frequencies $\gtrsim 2 f_p$ and lie on the signals for frequencies $< 2 f_p$. The lower frequency boundary of the e.l.f. hiss seems to be close to the local proton gyrofrequency of 243 Hz.

Also shown are whistlers exhibiting the fine structure related to the helium gyrofrequency reported earlier[2,3]. Note that the whistler to the left at about 2329.05 UT has fine structure such that the horizontal lines pass through the gaps, indicating interaction at a location where the helium gyrofrequency is 112.5 Hz, the same as for the e.l.f. hiss modulation, suggesting a relationship between the two phenomena. The same feature is seen on the whistler to the right at about 2329.10 UT for frequencies >2 kHz. Below 2 kHz the signal, or perhaps another signal, seems to be displaced a fraction of a second to the right and has fine structure leading to a helium gyrofrequency of ~100 Hz corresponding to $f_p' = 400$ Hz, suggesting a slightly higher interaction region than that for the other whistler. Horizontal lines at these harmonics pass through the gaps in the whistler, as before. This feature suggests attenuation of the whistler at the harmonics of the helium gyrofrequency. For the very faint whistler at about 2329.06 UT, however, the lines pass through the signals, suggesting enhancement at integral values of f_{He}. To be cautious, however, although the fine-structure frequency has been carefully scaled to $\lesssim 1.5\%$ accuracy here, a difference of 2.5% would shift the 20th helium gyrofrequency harmonic line ($5 f'_p$) by half a harmonic so that the gaps of the whistler in the box would lie between rather than on the harmonic lines. The phenomenon must, nonetheless, be related to the helium gyrofrequency, and for those examples with a large number of harmonics the very high order harmonic frequencies can be more accurately determined.

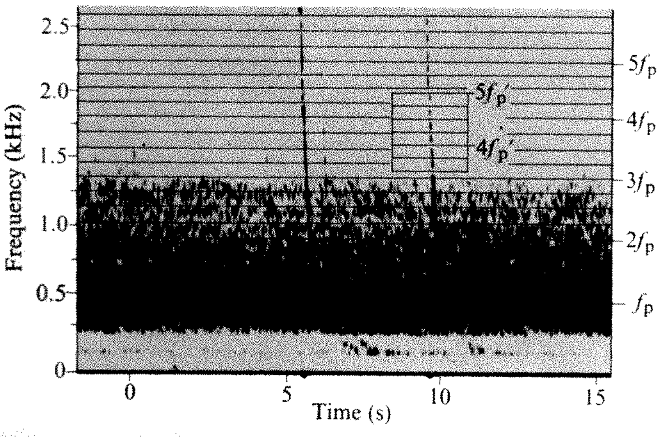

Fig. 1 e.l.f. hiss exhibiting modulation related to the helium gyrofrequency. The fine structure leads to a helium gyrofrequency $f_{He} = 0.25 f_p = 112.5$ Hz, which is greater than the local value of 60.7 Hz in the immediate vicinity of the satellite; the superimposed horizontal lines are at harmonics of f_{He}. Also shown are whistlers which may be related to the e.l.f. hiss.

Other examples of the e.l.f. hiss modulation gave values for the observed helium gyrofrequency which were approximately equal to and also less than the local value in the immediate vicinity of the satellite, depending on whether the satellite was somewhat near the plasmapause or outside the plasmasphere respectively. These observations are consistent with the statement by Taylor and Shawhan[4] that the propagation of e.l.f. hiss is downgoing outside the plasmapause and upgoing inside.

Since the e.l.f. hiss modulation provides a signature for the source region or regions where the modulation took place, it provides a means for studying the possible generation or interaction region of the e.l.f. hiss depending on whether the modulation took place at the same time as or after the e.l.f. generation. A preliminary calculation for the altitudes of the source regions, assuming wave propagation along the magnetic field of the Earth, gave ~2,300 km outside the plasmasphere, 500 km inside the plasmasphere and 1,400 km (the Isis II satellite altitude) near the plasmapause. Obtaining the observed gyrofrequency used to calculate the location of the generation or interaction region can be very complex since at times three or more components may be seen on the v.l.f. spectrograms, indicating a superposition of many e.l.f. waves from a number of source locations.

Work is in progress to analyse more examples of e.l.f. hiss modulation. The results of the analyses and more complete details of the items mentioned above will be published elsewhere. Further studies of the phenomenon, especially with data from satellites at other altitudes than the nearly constant Isis II satellite altitude of 1,400 km, may provide further clues as to the generation mechanism for e.l.f. hiss, the subject of some discussion in very recent papers[5-9].

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Thermoelastic instability in lubricated sliding between solid surfaces

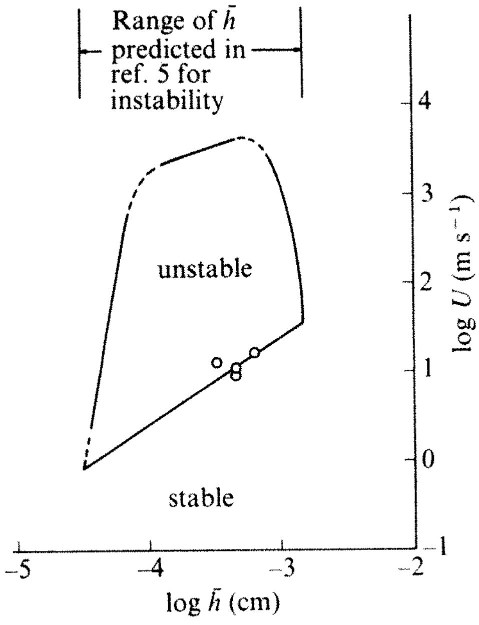
In the past few years, it has been recognised that, in certain conditions, thermoelastic instabilities occur in dry sliding contact between two solids[1-4]. We here report observations of the same phenomenon in the presence of a liquid lubricant which supports analytical predictions[5] of such an effect. Thermoelastic instability is caused by frictional heating and thermal deformation of the surfaces in contact, and alters the original contact configuration. Even if the surfaces are perfectly flat at the outset, instability will lead to a departure from flatness, ultimately resulting in patches of high pressure in the lubricant, with surrounding regions of low pressure and relative parting of the surfaces. This instability can lead to a malfunction of, for example, mechanical face seals.

If the instability leads to surface deformations, stability implies the suppression of any disturbance of the initial surface shape. The two regimes are demarcated by a threshold on which neutral stability prevails; on this threshold, a disturbance on either surface will be preserved without growth or decay. Several such thresholds exist in the case of a good thermal conductor sliding on an insulator[5]. Figure 1 shows an enclosed region, defined by the sliding speed and the film thickness, within which instability is predicted. The bottom edge of this region is the threshold of principal interest and the corresponding critical sliding speed is given by

$$U_{\mathrm{crit}} = \bar{h}\kappa\,(K_{\mathrm{M}}/\mu\alpha_{\mathrm{M}})^{1/2}$$

where $\bar{h}$ is the nominal film thickness, K_{M} and α_{M} the coefficients of thermal conductivity and expansion, respectively, of the conductor, μ the viscosity of the oil and κ is a wave number of the surface disturbance, being given by $2\pi/\lambda$, where λ is wavelength. It is also predicted that this instability will occur for only a range of $\bar{h}$, as shown in Fig. 1. These results were obtained from an idealised analysis where we tried to determine whether or not a small perturbation would grow on a surface that, initially, was perfectly flat. The analysis was not extended to the nonlinear effects that would eventually result from such a growth. But the experiments reported here show that such instabilities can occur in practice.

Fig. 1 Domain of instability for aluminium sliding on glass. The solid lines represent analytically predicted thresholds of ref. 5 for $\kappa = 0.24\,\mathrm{cm}^{-1}$; the dashed line corresponds to $\kappa = 0.48\,\mathrm{cm}^{-1}$.



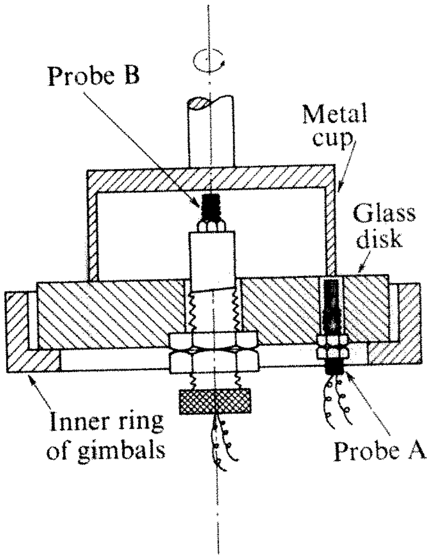


Fig. 2 Schematic diagram of experimental set-up, showing the location of the two measuring probes.

To demonstrate the effect, a circular metal cup with a flat, thin lip is rotated over a flat Pyrex disk (Fig. 2). The cup is rigidly held in a vertical spindle which has freedom of motion along its axis. The glass disk is flexibly mounted in gimbals. Placed in two holes in the glass, and rigidly affixed to it, are two eddy-current proximity probes. One of these (probe A) records the change of the distance between the lip of the cup, as it turns, and the tip of the probe. The other (probe B), located on the cup axis, records axial vibrations or bouncing of the cup as a whole. No attempt is made to control the film thickness, it being controlled by the operating conditions. When probe B indicates no bouncing of the cup, the time dependent output of probe A may be taken as the measurement of the contour of the surface passing over it. The wavelength λ will be an integral submultiple of the cup circumference.

At the outset, the surfaces of the cup and the glass are ground and polished to a mirror finish, with a waviness of $\sim 10^{-4}$ cm peak to peak. Oil is held in a shallow pool on the glass. The two probes are connected to a dual beam oscilloscope and each is also connected, in parallel, to a high impedance voltmeter for visual reading of displacements. As the cup begins to turn, the oil temperature rises, causing thermal expansion of probe A and, consequently, a slow drift in its reading, without affecting its dynamic response to the passing metal surface. For this reason, an initial running in period, at low speed, is allowed until the d.c. voltmeter reading for probe A stabilises. The rotational speed is then gradually increased in steps and time is allowed at each step for stabilisation of the d.c. voltmeter reading for probe A.

The zero shift with temperature makes it difficult to measure directly the time-average thickness of the liquid film. A technique has been adopted in which the d.c. voltmeter reading is allowed to stabilise, and then the rotation of the cup is suddenly arrested causing it to drop and rest on the glass. Voltages (d.c.) before and after this stoppage are noted and simultaneous photographs are taken of the oscilloscope traces. To this d.c. voltage drop is added the mean of the fluctuating (a.c.) component as indicated by the oscillogram for probe A before stoppage, to obtain $\bar{h}$ as defined in ref. 5. This procedure is carried out at several rotational speeds.

In Fig. 3 the upper trace corresponds to probe B and indicates d.c. voltages, the lower trace indicates the fluctuating (a.c.) component of the voltages from probe A. A tendency towards parting of the cup and the glass is represented by a rise in the

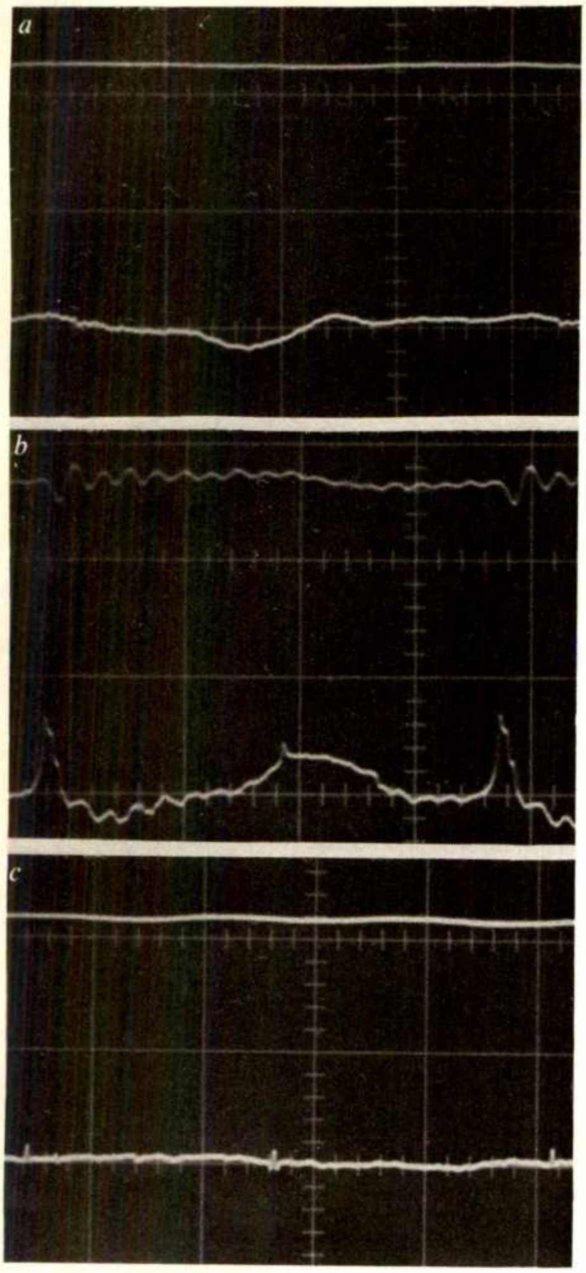


Fig. 3 Oscillograms at three different speeds. Upper trace corresponds to probe B, lower trace to probe A. One small vertical division corresponds to 0.004 mm. Each trace shows a little more than one revolution. *a*, Surface profile at 10.7 m s^{-1} speed, subcritical. *b*, Thermoelastically deformed surface at 13 m s^{-1} sliding speed, supercritical. *c*, Surface profile at 4.6 m s^{-1}, on slowing down from the deformed state; large deformations have disappeared; indicating that they were thermoelastically generated at the higher speed.

top trace and a drop in the bottom one, both to the same scale. Figure 3*a* shows the surface profile at a sliding speed a little below the critical. This profile was seen to change with time, with small thermally-generated bumps growing and moving on the surface, but with the surface wave amplitude remaining approximately constant. This represents a condition close to neutral stability, and has been observed repeatedly.

Figure 3*b* exhibits a dramatic deformation of the face at a somewhat higher speed, with large thermal asperities forming on the surface. This is taken as experimental evidence of the onset of the predicted instability. As a large asperity passes over probe A and dips into the slight depression above the probe, it sets off a vibration that is clearly indicated by the upper trace. This vibration is damped out until the next asperity passes over the probe and triggers another vibration. Figure 3*c* is taken at a lower speed after brief operation in the deformed condition of Fig. 3*b*. The asperities have all but disappeared, supporting the argument that they are not the manifestation of a permanent surface damage but are of thermal origin. Observation of the specimen after the experiment suggests that the asperities in Fig. 3*b* have broken through the oil film and are rubbing directly against the glass.

The qualitative aspects of these observations are repeatable, although the details of the deformed state change somewhat from specimen to specimen. For an aluminium cup of 7.62-cm diameter and 0.15-cm lip thickness, and with oil similar to SAE 10, the sliding speeds and film thicknesses corresponding to the onset of the deformed state are shown in Fig. 1 as data points. The solid line is the theoretically predicted threshold of instability for a λ of one full circumference ($\kappa = 0.24$ cm^{-1}), the dashed line for a κ of one-half the circumference ($\kappa = 0.48$ cm^{-1}).

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Linear dichroism of cations and anions in micellar solutions

WE here report a linear dichroism (LD) exhibited by isotropic chromophores in a condensed anisotropic medium and caused by anisotropic effective local fields. We shall call the effect Wiener dichroism (WLD) when the anisotropic reaction field arises from the presence of two (or more) different dielectric domains (phases in our notation) which are internally isotropic but which are separated by surfaces with non-spherical geometry. With a coloured cubic anion, like $Co(NO_2)_6^{3-}$, in the aqueous phase of a 20% solution of cetyltrimethylammoniumbromide (CTAB), known to contain rod-shaped micelles, a positive WLD is observed, while a cation, like $Co(NH_3)_6^{3+}$ yields negative WLD, when the micelles are oriented. This difference between cations and anions arises from the different average locations of the ions with respect to the cylindrical counter-ion layer surrounding the micelle. This is the first time a dichroism has been observed caused exclusively by local fields.

In the applications of linear dichroism spectroscopy[1-6] it has been accepted that the observed absorptions $A_{\parallel}$, $A_{\perp}$ in the macrosystem (for example a crystal) are directly related to transition probabilities in the microsystem (molecule) being proportional to the squares of the scalar products between the radiation electric field vector and the transition moments (sometimes after correction for effects on the polarisation of the field if the axes of the indicatrix or the linear dichroism ellipsoid do not coincide with the laboratory directions[7]). Though it seems intuitively clear that with anisotropic permittivity and hence different effective fields in different directions, $A_{\parallel}$ may differ from $A_{\perp}$, this has never been experimentally verified, even by molecular absorption techniques, and it has been simply assumed that the 'molecular dichroism' is proportional to the observed macroscopic dichroism.

We consider the case of elongated oriented particles (molecules, domains or crystallites) immersed in an isotropic medium. Wiener and other authors[8-10] estimated the macroscopic static permittivity ε in terms of the permittivity of the particle, ε_{2i} ($i = \parallel$ to the orientation axis and $\perp$, respectively), and of ε_1, the permittivity of the surrounding 'phase'. If the particle is approximated by an ellipsoid of revolution, the applied uniform field, $\mathbf{E}_0$, is modified inside the ellipsoid according to simple electrostatic theory, equation (1*a*), where L, the depolarising coefficient, depends on the geometry of the particle[9]. If the particles define the volume fraction f, the apparent average field, $\mathbf{E}=(1-f)\,\mathbf{E}_0+f\mathbf{E}_i$, yields ε_i of the system: equation (1*b*).

$$\mathbf{E}_i=\mathbf{E}_0/[1+L_i(\varepsilon_{2i}-\varepsilon_1)/\varepsilon_1] \qquad (1a)$$

$$\varepsilon_i=\varepsilon_1+f(\varepsilon_{2i}-\varepsilon_1)/[1+(1-f)L_i(\varepsilon_{2i}-\varepsilon_1)/\varepsilon_1] \qquad (1b)$$

This or similar expressions have been used to explain 'form birefringence' in oriented polymer solutions[11] and in crystals, for example, by the change in birefringence when water in a haemoglobin crystal was replaced by a salt solution (that is ε_1 changed) Perutz estimated the shape of the protein molecule[12], but a complex permittivity was not considered. By inserting $\varepsilon_{2i}=(n_{2i}+i\,k_{2i})^2$, $\varepsilon_1=(n_1+i\,k_1)^2$, eq. (1) may be solved with respect to the macroscopic refractive indices $n_{\parallel}$, $n_{\perp}$ and absorption coefficients $k_{\parallel}$, $k_{\perp}$ in terms of the particle coefficients, $k_{2\parallel}$, $k_{2\perp}$, the absorption of the surrounding medium, k_1, and the corresponding refractive indices, $n_{2\parallel}$, $n_{2\perp}$, n_1.

The molecular linear dichroism $k_{2\parallel}-k_{2\perp}$ can thus be correlated to the observed dichroism, $k_{\parallel}-k_{\perp}$ (relationship between k and the decadic absorption coefficient in eq. (2)). Wiener arrived at a similar conclusion 1912 but (probably because of the great length of his paper) his work has unfortunately remained largely unknown[8]. What happens if the particle is isotropic, that is $\varepsilon_{2\parallel}=\varepsilon_{2\perp}$? Eq. (1) then yields eq. (2), a linear dichroism which has its origin purely in the non-spherical geometry of the surfaces separating the dielectric phases, and which we call Wiener dichroism, WLD, in memory of its predicter (A is decadic absorbance, d pathlength).

$$\mathrm{WLD}=A_{\parallel}-A_{\perp}$$

$$A_i=k_i(4\pi\nu d/\log_e 10)$$

$$k_i=\frac{(1-f)k_1\{n_1^4+L_i(n_2^2-n_1^2)\,[2n_1^2+f(n_2^2-n_1^2)]+L_i^2(1-f)\,(n_2^2-n_1^2)^2\}+fn_1^3n_2k_2}{\{n_1^2+[f+(1-f)L_i]\,(n_2^2-n_1^2)\}^{1/2}\,[n_1^2+(1-f)L_i(n_2^2-n_1^2)]^{3/2}} \qquad (2)$$

$L_{\parallel}=L_{\perp}=1/3$ for a sphere, $L_{\parallel}=0$, $L_{\perp}=\frac{1}{2}$ for an infinite cylinder

If only the surrounding of the infinite cylinders absorbs ($k_2=0$) equation (2) may be approximated to $\mathrm{WLD}/A=-2f(1-f)[(n_2/n_1)-1]$, where A is the unpolarised absorption. This corresponds to the case of CTAB (20%) in an absorbing aqueous solution. Inserting $f=0.20$, $n_1=1.33$ (water), $n_2=1.40$ (CTAB) we get $\mathrm{LD}/A=-0.017$ (500–600 nm), which in sign and magnitude agrees with the observed effects of some isotropic chromophoric cations (Table 1) when the system is subjected to a shear gradient > 1,000 s^{-1} in a Couette cell[13]. The LD spectrum has the same shape as the absorbance spectrum, that is LD/A is approximately constant over the spectrum (Table 1).

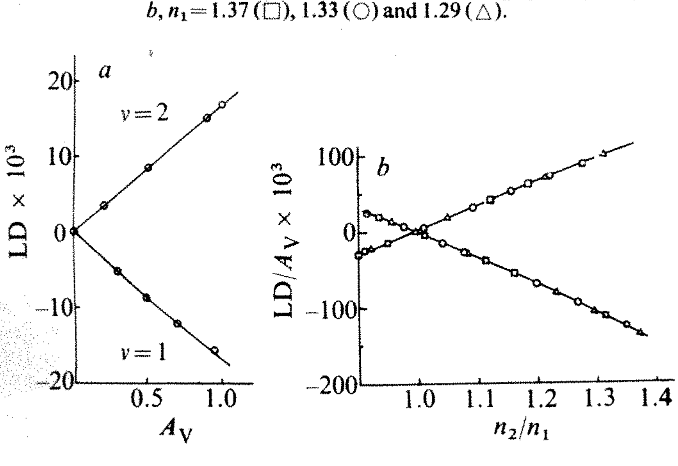

Fig. 1 LD/A according to equation (2), $f=0.20$; *a*, $n_2/n_1=1.4/1.33$; *b*, $n_1=1.37$ (□), 1.33 (○) and 1.29 (△).

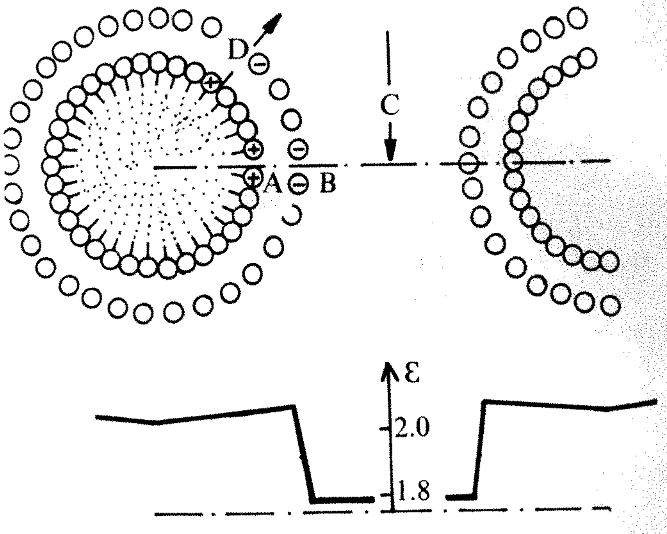

Fig. 2 Sketched structure in plane perpendicular to orientation direction of CTA$^+$ micelle cylinders surrounded by Br$^-$ cylinders, below schematic variation in permittivity.

Further, LD/A appears independent of the chromophore concentration, which is expected since the influence of the latter on n_1 is negligible at these concentrations.

The independency of LD/A of wavelength is evidence for completely negligible orientation dichroism ($k_{1\parallel}=k_{1\perp}$): thus if, for example, the trigonal Fe(dipy)$_3{}^{2+}$ had not been randomly orientated, positive and negative LD contributions should have appeared corresponding to the existence of mutually perpendicularly polarised transitions. Thus the non-cubic complexes also behave isotropically.

Finally an 'anomalous' behaviour of anions (Table 1), exhibiting positive LD, suggests an interesting possibility of using WLD for solution structural studies: a different LD/A implies a different average location with respect to the micelle cylinder (Fig. 1). The sign reversal may thus be explained by the sketched permittivity profile, which is expected if the bromide ions form a compact coaxial cylindrical mantle. The anions are preferably found in, or inside, the mantle (A), attracted by the solid positive charge, while the cations are outside (or in contact with) the bromide mantle (B). Figure 2 shows effects of different refractive indices in the cases of absorber inside and outside the mantle surface, according to equation (2). Obviously at fixed n_2/n_1 (the dispersions of n_1, n_2 have negligible importance in the present examples) LD/A is almost independent of A, and LD/A varies almost linearly with n_2/n_1.

Though not considered when averaging the fields in eq. (1), it is evident (at least with distances of the order of the wavelength) that the magnitude of the effects can be increased if the centre of gravity of the chromophore concentration is moved nearer the dielectric discontinuity surface, in agreement with the larger and more varying WLD of the anions. A complete

Table 1 Wiener linear dichroism of various chromophoric ions in CTAB–water solutions (20% CTAB) with almost complete micellar order, $d=0.1$ cm

Chromophore	Symmetry	Wavelength (nm) of LD_{max} and A_{max}	LD/A
$Co(NH_3)_6{}^{3+}$	O_h	340	−0.015
		470	−0.020
$Co(en)_3{}^{3+}$	D_3	340	—
		469	—
$Fe(dipy)_3{}^{2+}$	D_3	297	−0.013
		530	−0.011
$Fe(gmi)_3{}^{2+}$	D_3	552	−0.010
$Co(NO_2)_6{}^{3-}$	O_n	258	+0.020
		358	+0.025
$Fe(CN)_6{}^{3-}$	O_h	428	+0.036
$MnO^-{}_4$	T_d	310	+0.066
		525	+0.044
$CrO_4{}^{2-}$	T_d	270	+0.027
		365	+0.020

Concentrations varied between $A=0.1$–1.5 (dipy=dipyridyl, gmi =glyoxal-*bis*-*N*-methylimine, en=ethylenediamine).

theory should thus include some concentration profile function. Effects caused by the large electrostatic fields (C, D in Fig. 2) should also be considered: orientation dichroism by permanent or induced dipoles on the chromophores and anisotropically perturbed transition probabilities due to polarisability[14] of the electronic states participating in the absorption. It is important finally to investigate to which extent the WLD fields are consistent with different local field models[15-18].

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Dye sensitised zinc oxide: aqueous electrolyte: platinum photocell

TITANIUM oxide photocells, which are photochemically stable and decompose water into hydrogen and oxygen[1,2], have a low efficiency[3] and their spectral response is mostly in the ultraviolet, where the Sun's output is low. Other semiconductors such as cadmium sulphide or gallium phosphide, having smaller band gaps, are destroyed by light and their photocurrents deteriorate rather rapidly[4]. Photocurrents in dye sensitised photocells composed of aqueous electrolyte solutions and various semiconductor electrodes have also been studied[5-7]. All results obtained so far show that the dye sensitised photocurrents are very small, ~ 10^{-6}–10^{-9} A cm^{-2}, corresponding to a quantum efficiency $\lesssim 10^{-2}$. In this paper we report the successful construction of cells with a high power efficiency.

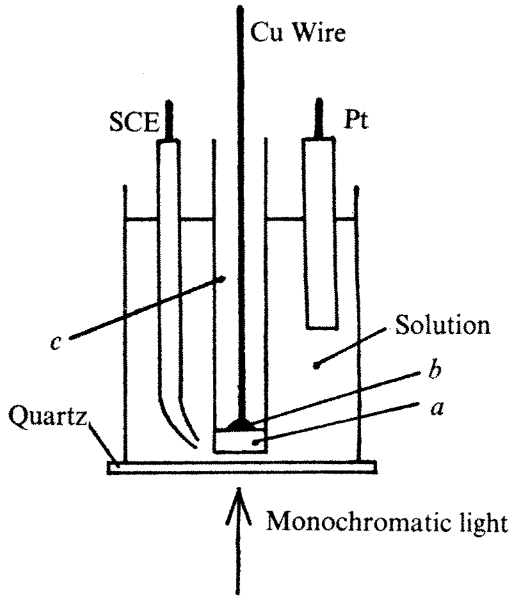
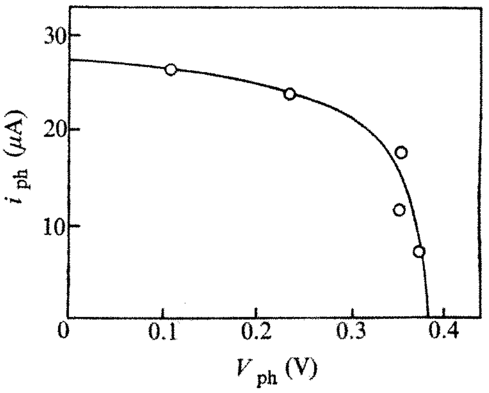


Fig. 1 The design of the photocell. *a*, ZnO sinter disk; *b*, indium contact; *c*, glass tube cemented to the electrode with epoxy resin. SCE, standard calomel electrode.

Most of the work on dye sensitised photocells by previous workers as well as in the early stage of our work was done with the electrodes dipped into aqueous electrolyte–dye solutions. Various experimental results obtained seem to show, as Gerischer and Memming pointed out[5,6], that only the dye molecules (or ions) directly adsorbed on the surface of the electrode cause the photocurrent, not those in the bulk of the solution. The poor photocurrents can then be attributed primarily to a small quantity of such a dye on the surface of the electrode. For instance, if the dye forms a monomolecular layer, the absorptivity by the dye is ~ 10^{-2}, and therefore most of the light passes through the dye layer without causing any useful effect.

We first tried to coat various insoluble dye films on the surfaces of the electrodes to increase the 'useful' absorbance, but this was unsuccessful, mostly because these films form electrical insulating layers, when they become sufficiently thick to appreciably absorb light. After these trials we succeeded in constructing a cell showing a high power efficiency. The electrode is a zinc oxide sinter disk ~ 1 cm in diameter, prepared from commercial high grade ZnO powder moulded by compression and heated at 1,300 °C for 1 h in air. The disk is then dyed by keeping it in a concentrated aqueous rose bengal solution. We found that the porosity and the dye-adsorbing capability of the ZnO sinter depend largely on the ZnO powder used and the method of sintering. Some adsorb the dye evenly and strongly and others do not. With the former, a high photocurrent efficiency could be obtained. The counterelectrode is a

Fig. 2 Curve of photocurrent (i_{ph}) against photovoltage (V_{ph}) for the ZnO photocell dyed with rose bengal.



plane platinum plate. The solution comprises 0.2 M Na_2SO_4, 0.13 M potassium iodide and 1.0×10^{-3} M iodine.

The cell design is shown in Fig. 1.

Figure 2 shows the photocurrent obtained across a variable resistance, *R*. The photocurrent on short circuit was 28 μA which corresponds to a quantum efficiency of 15% (the number of electrons passed per s divided by the number of incident photons per s). The open-circuit photovoltage was $\sim$ 0.4 V, and the maximum power (i^2R) was 6.2×10^{-6} W at $R = 20$ kΩ, with 4.1×10^{-4} W, of incident radiation, monochromatised at 563 nm, corresponding to a power conversion efficiency (power produced per s divided by incident light energy input per s) of 1.5%.

These values are at least one order of magnitude higher than our previous values (ref. 8, and H. Tsubomura *et al.*, unpublished), the photocurrent being also quite high compared with those reported before by other authors.

The high efficiency of our cell is probably due to two things. First, the zinc oxide sinter has a fairly low inner resistance, $\sim$ 100 Ω cm. Moreover, as it is porous, the overall quantity of the dye adsorbed on the surface of the electrode can be fairly large, with the thickness of the dye layer on the convoluted surface being kept rather small compared with those obtained by the coating method mentioned before (maybe $\sim$ 1–2 molecular layers). Thus, we get a large total absorption of light without producing undesirable insulating or energy dissipating effects. Second, the use of a $KI–I_2$ redox couple improves the cell in two ways. First, the iodide ion serves as an electron supplier to the photo-oxidised dye. Without I^-, or any other reducing agent, the photocurrent decays quickly due to an accumulation of the oxidised dye on the surface[8]. Second, the $KI–I_2$ couple (or, more correctly, $I^-–I_3^-$ couple) keeps the electrode potential of the counterelectrode low and the photovoltage nearly the same as under open-circuit conditions. In other words, the presence of I_2 in the solution prevents the accumulation of over-potential of the platinum electrode at the working condition. (It was confirmed that the dark current of this cell was negligibly small).

The photogenerated power of the cell decreased drastically as the I_2 concentration was lowered. At $[I_2] \leqslant 7 \times 10^{-6}$ mol l^{-1} the potential of the counter electrode shifts to the negative side, the size of the shift being the larger the higher the photocurrent. We studied the effect of adding other redox couples. With hydroquinone-quinone, for example, a similar *i–V* curve was obtained, but the $I^-–I_2$ couple has given the best result so far.

The merits of our present cell are: (1) The electrode material is zinc oxide sinter which is much less expensive than single crystal semiconductors. It is also very easily prepared in any large size. (2) The dye absorbs light in the spectral region where the solar energy is most abundant. (3) Further improvement of the characteristics of such cells seems promising—by studying optimum conditions for the method of preparation of the semiconductor and the choice of the redox couple, dyes and their concentrations.

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Photoelectrochemical energy conversion and storage using polycrystalline chalcogenide electrodes

WE report here on a major improvement in the conversion efficiency of corrosion-free photoelectrochemical cells (PECs) and on a novel extension of such cells which allows the storage of part or all of the converted energy *in situ* for subsequent use.

We have used, for example, a 2 cm^2 Cd–Se photoelectrode, prepared by the electrolytic codeposition of Cd and Se on a conducting base, followed by heat treatment in an inert atmosphere. Corrosion of the photoelectrode was prevented by using an aqueous or organic solution of sulphide in which some elemental sulphur was dissolved and from which air was rigorously excluded. Using a Cd–Se electrode, currents of 7–10 mA cm^{-2}, and open circuits, potentials from 450–560 mV were obtained in AM1 sunlight. Because of the large currents generated with these electrodes, the counterelectrode may limit the current, so that minimally polarised counterelectrodes with large surface areas, such as activated carbon, should be used. Part of or all the converted energy is stored in a controlled way in the system by the introduction of an electrode of porous silver:

$$2Ag + S^{2-} = Ag_2S + 2e^- \ (E_0 = -0.69 \text{ V}) \text{ (see ref. 1)}$$

All known stable PECs use semiconductors with a wide bandgap ($E_g > 2.8$ eV) as the photoactive electrode[2-4], thus severely limiting their practical use for solar energy conversion. The main difficulty in the use of semiconductors with bandgaps which are optimal for solar energy conversion ($E_g \sim 1.4$ eV) in PECs, is corrosion of the photoactive electrode, either directly or under the influence of light (unpublished). The aim of most workers in this field has been the photoelectrolysis of water[5-7] which we do not consider a practical proposition (see ref. 8). For the photoelectrolytic production of hydrogen from water only the O/O^{2-} redox couple can be used. If, however, the purpose is electricity generation, other redox couples such as S/S^{2-}, with a much lower redox potential, can be used (E_0 (O/O^{2-}) = 0.4 V; E_0 (S/S^{2-}) = -0.45 V; both in 1N basic solution). Therefore, the photoelectrode can be held at a much lower anodic potential, aiding in the prevention of corrosion. Besides Cd–Se, other semiconductors such as Cd–S,

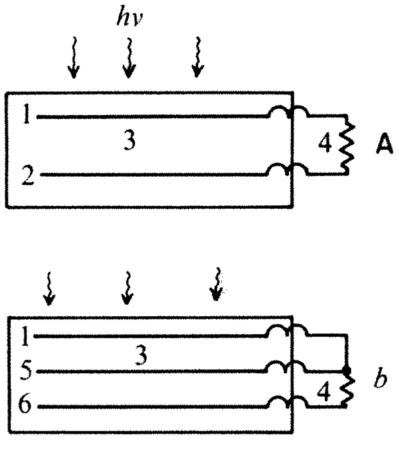


Fig. 1 PEC and PESC configurations: *a*, 2-electrode system (current direction in the light opposite to that in the dark, when used as storage cell); *b*, 3-electrode system (no current reversal between light and dark). 1, Chalcogenide photoelectrode; 2, inert counterelectrode or storage electrode; 3, S^{2-}/S solution; 4, load; 5, storage electrode; 6, inert counterelectrode.

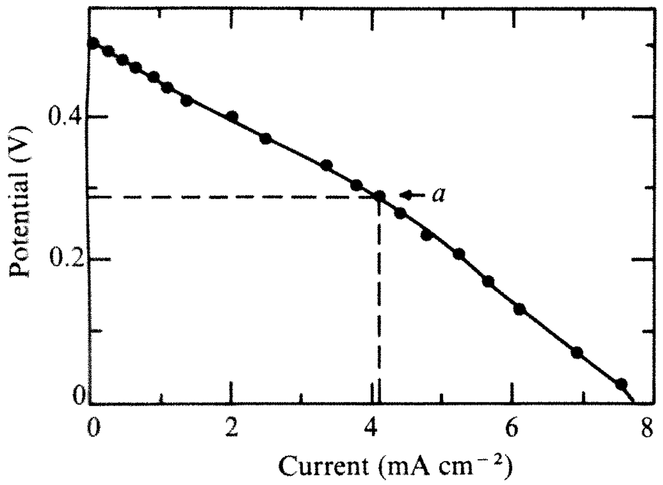
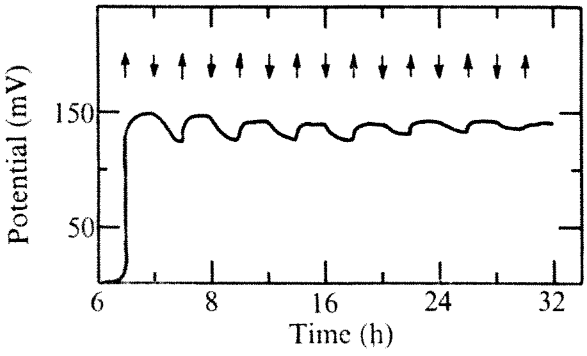


Fig. 2 Voltage–current behaviour of a PEC comprising a 1 cm^2 polycrystalline Cd–Se layer on Ti and a 4 cm^2 counterelectrode of activated carbon, both immersed in a 1M solution in OH$^-$, S^{2-} and S. Illumination by a 1A tungsten-iodine projector equivalent to AM1 sunlight. *a*, Maximum power.

Cd–Te (n- and p-type), Zn–Se or Bi_2S_3 are also stable as photoelectrodes in a polycrystalline form in such conditions.

Figure 1 shows two configurations of a chalcogenide PEC, or photoelectrochemical storage cell (PESC). Such cells are stable over periods of months (longer term trials are in progress). The absence of corrosion has been demonstrated in two ways. A 1 cm^2 polycrystalline Cd–Se photoelectrode on Ti was prepared by the electrolytic codeposition of Cd and Se from an acidic aqueous solution of $CdSO_4$ and SeO_2 using 4.5 C per electrode side, yielding a Cd–Se layer of about 7.5 μmol. The electrode was immersed in 10 ml of 1M S^{2-}/S solution, and illuminated for 15 h. Enough current (270 C) was passed to corrode all of the Cd–Se layer about 200 times, but the performance of the cell did not diminish. Moreover, the solution in the cell was analysed for Cd, before and after illumination, using atomic absorption spectroscopy. No change in the Cd content of the solution ($< 2 \times 10^{-6}$ M) was observed. During the first few hours of illumination, a thin layer of the chalcogenide (for example, Cd–Se) will undergo partial S/Se substitution if non-sulphide chalcogenides are used. Thus, a thin sulphide layer is obtained near the surface exposed to the electrolyte. Electron microprobe studies show that the S/Se ratio, in a Cd–Se electrode that had been illuminated for several hours, was about 1:1 in the top micrometre of the layer. Also, X-ray diffraction showed the presence of some CdS in the Cd–Se layer. The spectral response of the electrode remains essentially that of the original chalcogenide, however, indicating that only a top layer of Cd–Se was transformed into CdS.

Fig. 3 Voltage against time for a 3-electrode PESC without membrane. The PESC consists of the components of the cell of Fig. 2 to which an Ag–Ag_2S storage electrode is added in the configuration illustrated in Fig. 1*b*. Load, 680 Ω; ↑, light on; ↓, light off. Before the experiment the cell was completely discharged. The overpotential for sulphur reduction at the photoelectrode is sufficiently high to ensure that almost all of the current flows from the storage electrode over the load to the counterelectrode in the dark and not back to the photoelectrode.



The performance of a Cd–Se PEC is illustrated in Fig. 2. It can be seen that the potential drops rather severely under load. This is attributable to resistance losses in the system such as those in the photoactive layer, those in the electrolyte (which depend on the cell geometry, among other things), polarisation losses at the counterelectrode, and losses at the semiconductor–metal contact. The necessary improvement in the conductivity remains as a major challenge in obtaining a practical PEC.

Results obtained using a 3-electrode PESC are shown in Fig. 3. Storage efficiencies are low when the storage electrode is in the same solution as the other electrodes. If a cation-specific membrane is used to separate them, and care is taken to exclude sulphur from the storage compartment, storage current efficiencies of up to 90% can be obtained with a silver electrode. It is possible to use other systems, such as FeS–Fe_2S_3 and Sn–SnS. By using $Fe(OH)_2$–FeO(OH) as a storage electrode a PESC with an oxide electrode and O/O^{2-} couple can be constructed.

A report to be published elsewhere will consider the chemical and physical basis of the stability and storage capabilities of these cells, their spectral response, potentiostatic behaviour and the analogy between the semiconductor–electrolyte boundary and Schottky and Bardeen semiconductor–metal contacts, particularly with regard to surface states.

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Oldest organic remains of boring algae from Polish Upper Silurian

REMNANTS of cells and branched filaments of carbonate boring (endolithic) algae have been found in the Upper Silurian sedimentary rocks of eastern Poland. The material was obtained from a borehole at Widowo, near Bielsk Podlaski at a core depth of 524–525 m. The age of the sediments at a core depth of 545–585 m was determined as the Upper Silurian (Wenlockian), based on tabulate corals and stromatoporoids. The coral–stromatoporoid assemblage corresponds to the Jaagarahu Stage of the Silurian of Estonia[1,2]. It includes tabulate and heliolitoid corals *Coenites juniperinus* Eichwald, *Palaeofavosites collatatus* Sokolov, *P. frivolus* Klaaman, *P. tersus* Klaaman and *Heliolites decipiens* M'Coy[3], and stromatoporoids *Densastroma pexisum* Yavorsky and *Stromatopora impexa* Nestor (Kazmierczak, unpublished).

The boring algae occur in poorly sorted, coarse calcarenite, comprising mostly fragmented crinoid columnals and finely broken brachiopod and mollusc coquina. Pieces of the dasycladaceous alga *Rhabdoporella* Stolley, and bundles of *Girvanella* Nicholson and Etheridge are common. A few fragments of trepostome bryozoans and solenoporacean algae have also been observed.

Carbonate boring algae have been found exclusively within the crinoid columnals. Since they penetrate the columnals from all directions including the lumen and suture sides, it is concluded that their colonisation took place after the disintegration of the crinoic stems, but before their burial and the subsequent consolidation of the sediments.

The algae were studied *in situ*, in petrographic thin sections (Fig. 1*a*). The borings begin at the surface and penetrate perpendicularly into the interior of crinoid columnal fragments. No epilithic filaments have been observed. The endolithic filaments are composed of torulose, isodiametric, or slightly elongated cells 10–30 μm wide (mean $\pm$ s.d. $= 18.1 \pm 4.6$, $n = 100$), and 12–35 (22.2 ± 6.0, $n = 100$) μm long. They are repeatedly and densely branched, forming small bushes, 80–200 μm in diameter (105.6 ± 35.0, $n = 20$), within the substrate. Branching in all directions is lateral or subdichotomous. The cellular outlines are well defined by a brown, slightly shrivelled wall structure. One or two irregularly shrunken or flattened bodies 4–10 $\times$ 6–14 μm ($6.3 \pm 2.5 \times 8.1 \pm 4.3$, $n = 20$) are found within most cells.

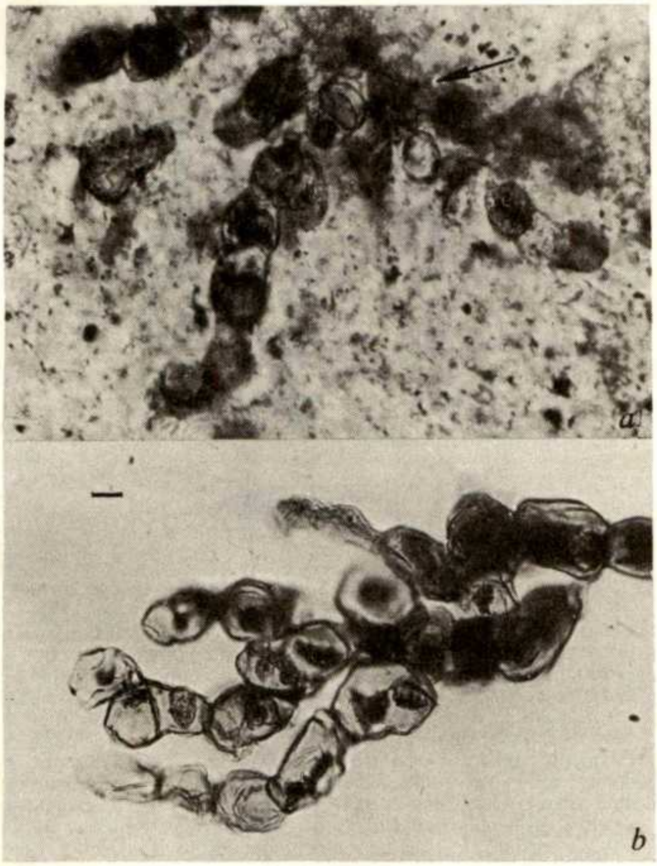

Fig. 1 Branched filaments of the Upper Silurian boring green alga discussed here: *a*, boring filaments *in situ*, penetrating a columnal crinoid fragment, petrographic thin section, transmitted light (arrow, substrate surface); *b*, filaments extracted with dilute HCl. Scale bar, 10 μm.

Algal filaments have been extracted by gradual dissolution of the carbonate matrix with 0.1 N HCl, and mounted in glycerol. They remained cohesive, although rigid and fragile (Fig. 1*b*). Selected filaments have been embedded in Araldite and sectioned for transmission electron microscopy (TEM), with and without osmium tetroxide, lead citrate and uranyl acetate treatment. Application of osmium tetroxide did not affect the image, but lead citrate and uranyl acetate increased the contrast.

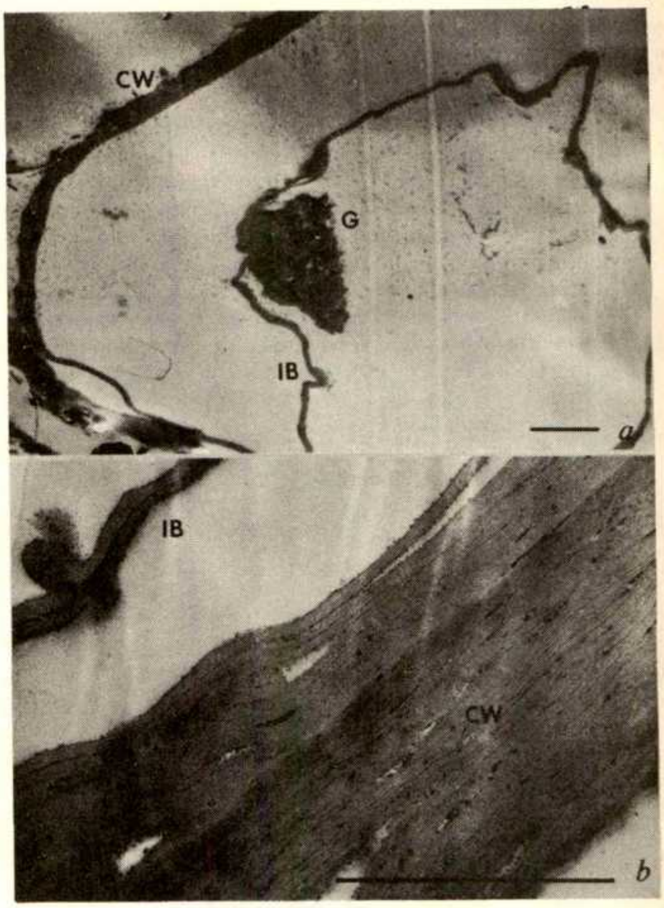

Fig. 2 Preserved cellular structures of the Upper Silurian boring green alga: *a*, section through the cell (×9,200); *b*, detail, multilayered wall structures (CW, outer cell wall; IB, internal body outline; G, granular aggregate), (×36,800). Scale bars, 1 μm.

At the TEM level, the outer walls, 300–1,200 nm thick, revealed a multilayered structure with distinguishable, individual, layers 10 nm thick (Fig. 2*a* and *b*). Packets of layers are frequently folded or detached. Cross walls have similar structures, but are generally thinner (100–400 nm). They meet with the longitudinal walls by forming a triangular space. No plasmodesmic connections have been identified on the cross walls. The outlines of the internal bodies are less than 100 nm thick. They also have a layered substructure and an electron density similar to the external and cross walls, but they are more convoluted and shrivelled. Occasional thickenings extend towards the lumen of the internal bodies. Within the internal bodies there are occasional isodiametric or flattened aggregations of electron dense masses with granular substructures. The ultrastructure of the preserved organic residue permits only an identification of the multilayered cell walls. Subtle structural features such as plasmodesmata, considered an important taxonomic marker[7], are either not present or have become diagenetically obliterated. No closer identification of intracellular features has been possible.

Borings of endolithic algae are known as far back as the Ordovician[4]. The significance of the present finding lies in the preserved organic parts which allow determinations of cell sizes and shapes, the positions of cross walls and modes of branching, and the fine structures of the organic residues. The preserved features, along with comparisons with modern boring algae, identify the remains as Chaetophorales.

Endolithic algae are common within the illuminated portions of modern marine carbonate rocks and skeletal fragments in sediments[5]. Extant endoliths are represented by a few highly specialised prokaryotic (cyanophytes) and eukaryotic (chlorophytes and rhodophytes) algae. The

Silurian fossil described here compares well with septate endolithic chaetophoralean chlorophytes such as *Phaeophila, Eugomontia* and *Entocladia*[6], but cannot be directly identified with any of the modern genera. Morphologically, it is closest to *Entocladia* (or some growth forms of *Eugomontia*), which does not bear bristles (chaetae). The torulose cell shape and the mode of branching are similar to various epilithic (*Gongrosira*) as well as endolithic chaetophorales. Thus, it is conceivable that this Silurian endolith represents an ancestral type of some Holocene endolithic green algae.

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Fossil iron bacteria may be preserved in Precambrian ferroan carbonate

CONCLUSIVE evidence for microbial participation in the genesis of the massive sedimentary iron formations from the Precambrian is fragmentary. Structures resembling fossilised bacteria have been reported previously, and they tend to resemble modern iron bacteria such as *Sphaerotilus, Gallionella* and *Metallogenium*[1,2]. The atmosphere at that time is believed to have been almost devoid of oxygen[3]. The concentration of atmospheric oxygen probably ranged from 0.0001 to 0.001 % of the present atmospheric level, and may have remained at this level for 2 × 10^9 yr after the emergence of algal photosynthesis[4]. The fossils described in this paper were found during a study of the mineralogy of the Lower Proterozoic Brockman Iron Formation (Hamersley Group) of Western Australia (about 2 × 10^9 yr old[5]). Two specimens (PM9 and PM24) collected from the Whaleback Shale Member, at Wittenoom Gorge, were identified as dolomitic chert by X-ray diffraction. Optical microscopy showed the presence of fibrous stilpnomelane, and the staining technique suggested by Dickson[6] distinguished the dolomite as ferroan.

During our study of the carbonates, it was observed that some ferroan dolomite crystals showed filamentous structures on or near the surface of the crystals (Fig. 1*b*–*e*). They were stained deep blue, showing the presence of ferrous iron. The morphology of these filaments resembles the modern iron bacterium *Sphaerotilus*.

A weakly acid solution of potassium ferricyanide $K_3Fe(CN)_6$ was used to stain petrographic thin sections (0.2 g $K_3Fe(CN)_6$ in 100 ml 1.5% HCl)[6]. $K_3Fe(CN)_6$ reacts with ferrous iron to give a deep blue—Turnbull's blue—precipitate of ferrous ferricyanide:

$$3FeCl_2 + 2K_3Fe(CN)_6 \rightarrow Fe_3[Fe(CN)_6]_2 + 6KCl \qquad (1)$$

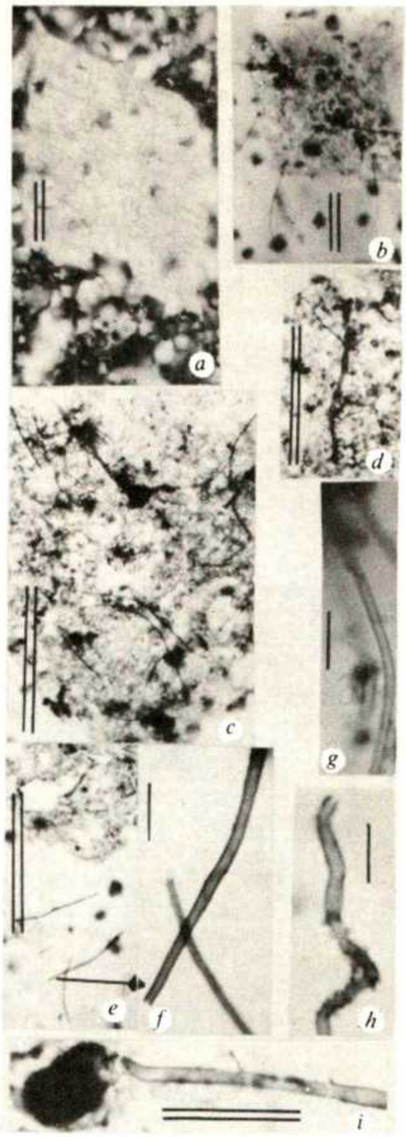

Fig. 1 Except where otherwise stated, all the illustrations are photomicrographs taken on a Zeiss photomicroscope in the Geology Department, Royal School of Mines, London, from thin sections of Whaleback Shale sample PM24. *a*, Dolomite rhomb before staining under crossed nicols. *b*, Dolomite rhomb after staining showing filaments on the surface of the crystal. *c* and *d*, Filaments on the surface of the crystal, some attached to rounded bodies. *e*, Filament detached from the crystal during coverslip mounting. Arrow indicates same filament in *f* at higher magnification. *g*, Modern specimens of *Sphaerotilus* from Silwood Lake, Berkshire. *h*, Isolated filament for comparison with *g*. *i*, Rounded body to which the filament has been attached, mainly composed of ferrous iron. Single marker bar indicates 10 µm. A pair of marker bars indicates 100 µm.

The section was first cleaned with 'Teepol' to remove all grease or immersion oil, dipped in a beaker containing the above stain, and allowed to react for 45–60 s. The slide was removed, and immediately rinsed with distilled water. The stain is on the surface of the minerals in the form of a precipitate, and may easily be destroyed if touched. Thorough washing with water is necessary to remove all the acid; otherwise carbonate crystals (especially calcite) would dissolve. Care is also required in the use of water, as prolonged contact of the precipitate with water renders it soluble.

Isolated filaments (*f* and *h*) were obtained when mounting the coverslip. A drop of glycerine was applied to a thoroughly water-washed, stained slide, to preserve it for a longer period of time. The slide was then warmed to make the glycerine less viscous, and the coverslip put in place. During this operation, some of the filaments became detached from the substrate and moved away to a new position free of the matrix.

Almost all the filaments I observed were attached to or lying on the surface of ferroan dolomite crystals, and each filament was filled with ferroan carbonate. No filaments were found in the rest of the matrix of the rock. The length of the filaments, on average, was 100 µm, and the fossils show no apparent morphological distortion, except that some of them had been broken during coverslip mounting. Many of the filaments seemed to be attached to a round body mainly composed of ferrous iron, to judge from the intensity of the staining (Fig. 1*i*).

Before staining (for details, see Fig. 1) ,the filaments in the slide were invisible (Fig. 1*a*). This may mean that they were preserved as carbonate moulds formed during early diagenesis. This is an example of an authigenic preservation process[7], involving early cementation in soft sediment, usually by iron and carbonate compounds—in this case, ferroan dolomite—preserving the configuration of organic parts. The process results in the preservation of structures of delicate shape and form.

The fossil material was compared morphologically with modern species of *Sphaerotilus*, collected from water samples from Silwood Park Lake, Ascot, Berkshire, UK A few drops of stain were allowed to react with the modern sample, and a similar colour change was observed (Fig. 1*g*). The morphological agreement is self evident when Fig. 1*g* (modern bacteria) and Fig. 1*f* and *h* (isolated fossil filaments) are compared. Somewhat similar structures have been detected preserved in haematite and chert of Tertiary age[8], and from other Precambrian iron formations from Canada[9], and from Western Australia[10]. It has been suggested that these other structures[8–10] represent the remains of fossil iron bacteria. Because of the strong morphological similarity between the stained ferroan dolomite filaments and modern *Sphaerotilus*, I suggest that the structures discussed here also represent the remains of Precambrian iron bacteria.

It might be argued that these tubular structures have formed as the result of boring by algae and fungi, but one would expect boring algae and fungi to be generally distributed throughout the rock. This may be ruled out, however, in the present case, because the filaments are invariably associated only with the ferroan dolomite crystals, and are not generally distributed. Furthermore, algal and fungal filaments are generally much larger, and may often be septate. Septa were not observed in my material. If the structures represent borings, then a trail of mucilaginous material might be expected around the filaments. None were observed, and no trails or 'borings' (if such they are) seem to connect up the individual and spatially isolated ferroan dolomite crystals. Note also that if the filaments do represent the remains of iron bacteria, that present-day examples are always associated with clots of colloidal iron hydroxides; it may be suggested, therefore, that the ferroan dolomite crystals represent the diagenetic products of such clots.

Statements have been made in the past that "bacteria do not have hard parts that can be fossilised, so there is little to be learned directly about their evolution"[11]. In view of the application of this staining technique to carbonate rocks, this statement must be modified. Also the technique, or similar methods, must be extended to other forms of preservation—such as in pyrite, in limonite, in phosphate, in silica, and in calcite. As well as providing evidence for the presence of iron bacteria in ancient rocks, the technique offers the possibility of discovering previously undetectable microfossils from many different sedimentary environments in the Precambrian that are at present considered to be barren.

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Viable bacterial spores recovered from an archaeological excavation

THERMOPHILIC actinomycetes grow rapidly and sporulate profusely in natural high temperature habitats such as composts, stored fodders and cereals. In these habitats can be found large numbers of *Thermoactinomyces* spp. endospores which exhibit longevity and extreme resistance to adverse conditions. Such spores have been recovered from lake muds and have been estimated to be more than 1,000 yr old, but it is important to have more accurate dating. For this purpose we have examined occupational debris from a Roman archaeological site which has been shown to be rich in plant debris and has been accurately dated from associated artefacts. Our findings show a strong correlation between viable spore count of *Thermoactinomyces* spp. and the nature of the substrate. We have also shown that it may be possible to undertake qualitative and quantitative analyses, using microbial taxa with specific environmental requirements, for archaeological interpretation. Furthermore, the study of evolutionary trends within the genus *Thermoactinomyces* is now possible.

Pre-Hadrianic buildings (85–125 AD) outside the conjectured area of the Roman fort and *vicus* at Vindolanda, Northumberland, UK (grid reference 35(NY)/771662; altitude 160–165 m) were located by chance while Mr R. Birley, director of archaeological excavations, was preparing a drain in late 1972. The digging season in 1973 revealed the full potential of this find: within the occupational debris uncovered were two distinct layers which have since been dated as (85–) 90–95 AD and 95–105 (–110) AD=strata I and II respectively in Fig. 1, each of which is characterised by a high organic content (61–89% by dry weight). Two further major occupational levels—strata III and IV in Fig. 1—are superimposed on these layers and can be dated between (105–) 110 and 125 AD; minor debris or occupational levels, for example, stratum IIa in Fig. 1, were encountered occasionally.

The occupational debris levels are sandwiched between compacted clay levels, indicating that the floor of the particular building had degenerated to such an extent that a new and more permanent floor was the answer to such problems as poor sanitation and a rising water table. A new floor may have coincided with partial or total reconstruction and/or replacement of the superstructure, for the basal plans of the successive building programmes provide a complex picture. The walls of the buildings were composed essentially of birch uprights interspersed with seasoned and fashioned oak; the whole was interwoven with birch branches, often supplemented by other tree species (for example, hazel, oak and willow).

The rooms so far excavated, which cover 30 m², were possibly put to various uses during the 40–45 yr of occupation. rooms which probably served in the first instance as living-quarters later had other functions such as bracken storage, tanning and leather-working, animal housing and the disposal of occupational debris. An earth and rubble

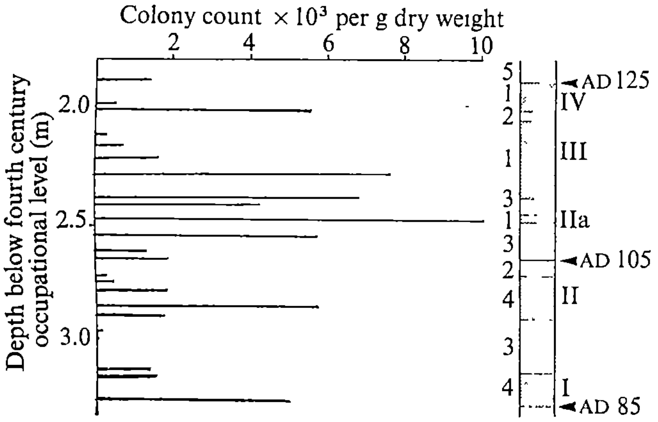

Fig. 1 Recovery of thermoactinomycetes from occupational debris and compacted clay strata at Vindolanda, Northumberland. Diluted samples were spread on the dried surface of a selective isolation agar containing novobiocin and cycloheximide[7], and incubated at 50 °C for 48 h. I–IV, Major occupational strata. Composition of strata: 1, highly organic, composed mainly of straw and bryophytes; 2, less organic, with high percentage of mineral soil; 3, compacted clay; 4, highly organic, composed mainly of bracken, straw and bryophytes; 5, earth and rubble infill.

infill around 125 AD delimits the end of this phase of occupation; the site was redeveloped from the late second century through to the fourth century.

The clay compactions, between the different phases in the pre-Hadrianic occupational levels, have created pockets of chemical conditions which have preserved a wide range of natural and man-made materials representative of the period. Preservation is due mainly to the anaerobic conditions, probably enhanced by a rising water table, and to chemicals derived from the medium. The latter is highly organic, being composed of bracken[1], straw, bryophytes[2], puff-balls[3], hazel and walnut shells, acorns, gorse pods, leaves, stems (mainly heather) and twigs (mainly ash, hazel, oak, pine, rowan and willow), as well as a wealth of animal material which includes a considerable number of stable-fly puparia, bones, hairs, feathers, oyster and mussel shells, and excreta (part of which is human in origin)[4]. Tannins, derived from many of these components of the medium and from the considerable quantity of leather material present, are anti-bacterial in nature. And tannins may have been produced purposely for leatherwork. This is suggested by the extensive number of leather offcuts present and the urine-impregnated medium, which may have resulted from the collection of urine for tanning. Vivianite is common, and the enormous quantity of bones present in the deposits contribute phosphate. *p*H measurements of all deposits so far examined are remarkable for their homogeneity, all being within the limited range of 5.3–5.6.

As well as leather boots, shoes, sandals, clothing and offcuts, this medium has preserved a unique collection of writing tablets made from thin slivers of wood and bearing carbon-based ink writing, together with considerable quantities of cloth.

An examination of the levels of occupational debris for artefacts and macro-subfossils and other environmental material is being supported by microbiological analyses. Although palynological investigations are just beginning, the recovery of viable bacterial endospores from the complex media described above is considered here.

The pre-Hadrianic occupational layers seemed to be an ideal substrate in which to look for *Thermoactinomyces* endospores since those of *T. vulgaris* have been isolated from present-day bracken litter[5], are commonly associated with stored cereals and straw[6] and exhibit longevity[7].

Sections about 13 cm long (about 700 cm³ in volume) were cut from a freshly exposed vertical face of an excavation trench wall. These were stored in tightly sealed polythene jars at room temperature. Samples for microbial analysis were removed from the centre of each section after slicing it from top to bottom with a sterile knife. High numbers of *Thermoactinomyces* spp. were recovered from the occupational layers and smaller numbers from the compacted clay between these layers and from the infill above the deposits (Fig. 1). Since stratum I can be dated between 85 and 95 AD, there is strong evidence that spores have survived 1,890 yr. The chemical conditions of the deposits have contributed to the survival of viable *Thermoactinomyces* endospores. The persistence of biodegradable materials in these deposits suggests low or non-existent microbial activity brought about by anaerobic conditions, a high water table and a low temperature. There is a highly significant correlation between the colony count and the presence or absence of occupational strata (Fig. 1).

It seems therefore that counts of microbial taxa with specific environmental demands may prove valuable in archaeological interpretation. In the case described above, the high temperature and oxygen requirements of *Thermoactinomyces* spp. reflect the prevailing environmental conditions within the organic media before the suppression of endospore germination by encapsulation of the deposits.

Thermoactinomycetes belonging to the species *T. vulgaris*, *T. sacchari*, *T. dichotomica* and *T. candidus* have been identified among the isolates[8]. Taxonomic investigations have also revealed that the relative frequency of the *Thermoactinomyces* spp. differed markedly between the pre-Hadrianic deposits and recent soil strata. Furthermore, strains of both *T. vulgaris* and *T. dichotomica* are sometimes found within the deposits which differ from those considered typical of the currently recognised species[8]. The palaeogenetic implications of these findings are being investigated in more detail.

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Accumulation of cadmium by the American oyster, *Crassostrea virginica*

CADMIUM is a major environmental pollutant potentially harmful to health, and if the sea becomes polluted with this metal there could be a reduction in extensive sources of food[1]. Seafoods constitute a source of cadmium in the human diet[2] and in view of abundant evidence that shellfish accumulate trace metals[3–6], it is important to investigate cadmium pollution. People have become ill from cadmium poisoning after ingesting foods containing concentrations of 13–15 μg g^{-1} (13–15 p.p.m., ref. 7). We now report that adult oysters reared in seawater containing 0.005 p.p.m. cadmium accumulated up to 10.75 p.p.m. in 40 weeks. This accumulation, plus cadmium

Table 1 Cadmium in whole meats of *Crassostrea virginica* after exposure to 5 parts per 10^9 Cd^{2+} for 40 weeks (November 1972 to August 1973) in flowing unfiltered seawater

Date	Time (weeks)	Mean biweekly temperature (°C)	µg Cd per g wet weight		µg Cd per g dry weight	
			Control	Experimental	Control	Experimental
1973						
November	0	10.0	2.72	2 72	11.83	11.83
December	4	4 3	2.48	3.60	13.10	21.75
1974						
January	8	2.8	2.58	4.02	15.57	22.00
February	12	3.2	3.11	4.14	19.35	24 90
March	16	4 3	2.10	4.96	13.42	28.55
April	20	7.2	2.62	5.29	13 60	27.59
May	24	11.1	2.81	4.76	16.75	33.77
June	28	16.4	1.93	5.71	10.85	39.22
July	32	20.2	1.65	9.10	12.19	63.27
August	36	21 1	1.76	10 37	17.47	85.83
August	40	22 6	1.69	13.57	12.55	104.91

naturally present, brought the concentration of cadmium to 13 p.p.m. in the soft tissue, which represents a potential health hazard if oysters constitute a major item of the diet.

Three-year-old oysters, *Crassostrea virginica*, were obtained from Long Island Sound in October 1972. The mean shell length was 10.2 cm with a mean whole wet meat weight of 8.1 g. The animals were acclimatised for 1 month, in six fibreglass troughs, each measuring 3.75 m × 30 cm × 25 cm deep and supplied with flowing unfiltered seawater (30‰ salinity, ambient temperature of 1.5 °–23 °C) at a rate of 16 l min^{-1}. Each trough held 50 oysters on a false bottom consisting of polyethylene grids resting on polyvinylchloride pipe about 2.5 cm above the bottom.

After acclimatisation of the oysters, three troughs received a cadmium chloride solution ($CdCl_2 \cdot 2.5\ H_2O$) and three troughs served as controls. The seawater–cadmium stock solution contained 0.016 mg Cd per ml and was pumped at the rate of 5 ml min^{-1} to give, when mixed with incoming seawater entering at a rate of 16 l min^{-1}, a calculated concentration of 0.005 p.p.m. cadmium. Samples of water were taken from the troughs weekly, concentrated by a Chelex resin technique developed by Dr Earl Davey of our laboratory and assayed by atomic absorption spectroscopy. A range of values from 0.0047 to 0.0053 p.p.m. was found in the seawater from the experimental troughs and 0.0001–0.0002 p p.m. was found in the control samples. Throughout this study, the animals received no supplementary food because the seawater supplied to the troughs apparently contained sufficient food organisms. Mortality was negligible in both control and experimental groups.

Every 4 weeks, five oysters were removed from the experimental and control groups. They were shucked and the shell liquor and body fluids were retained with the whole wet meats. The meats were weighed, dried at 100 °C for 24 h and ashed at 425 °C for 18 h. After cooling, 10 ml of concentrated HNO_3 was added to the ashed samples which were then dried at 100 °C for 24 h and ashed for 18 h. When necessary, ashing and treatment with HNO_3 were repeated until a clean white ash was obtained. The final ash was dissolved in 5% HNO_3 and analysed for cadmium by atomic absorption spectroscopy. The results were expressed as µg of cadmium per g (p.p.m.) for both wet and dry weights.

A mean of 2.72 µg cadmium per g wet weight was found in oysters immediately after collection. Concentrations of cadmium in seawater at the sampling site were 0.0001–0.0002 p.p.m. Although the dry weight data show that *C. virginica* can accumulate cadmium regardless of the time of year, the rate of accumulation in summer (July to August) was more than double that of the winter and spring (Table 1): at this time the warmer seawater supported a greater metabolic rate so that more seawater passed over the gills[8]. From July 4 to August 29, the experimental oysters accumulated 45% of the cadmium present by the end of 40 weeks (Table 1). Oysters exposed to 0.0005 p.p.m. cadmium for 40 weeks from November 19 to August 29 accumulated a mean of 10.75 µg cadmium per g wet weight (Table 1).

Although we have observed that apparently unpolluted seawater contains less than 0.001 p.p.m. of cadmium, values equal to and exceeding 0.01 p.p.m. have been recorded in seawater and freshwater[9,10] and also in waters from which oysters have been collected[11]. The high level of cadmium contamination in estuarine waters is exemplified in Corpus Christi Harbor where as much as 0 078 p.p.m. has been reported[12]. Therefore, we felt that exposure of oysters to 0.005 p.p.m. cadmium was realistic because such concentrations can be found in the marine environment.

Increased cadmium concentrations seem to be due mainly to trace metal entering estuarine or coastal areas from industrial effluents and rivers. Ratkowsky *et al.*[6] found a close correlation between proximity to heavily urbanised areas and the concentration of metals in oysters: those growing near urban areas had the greatest concentration of one or more metals. Shuster and Pringle[5] found concentrations of 0.1–7.80 p.p.m. cadmium (mean 3.10 p.p.m) in whole wet meats of oysters sampled along the Atlantic coast of the USA. The freshly collected oysters we used contained between 1.78 and 3.78 p.p.m. cadmium wet weight (mean 2.72 p.p.m.).

Table 1 shows that oysters exposed to 0.005 p.p.m. cadmium for 40 weeks accumulated concentrations of cadmium (13–15 p.p.m. wet weight) that could have the same effect on man as foods containing 13–15 p.p.m. (ref 7). The emetic property of cadmium in excess of 13–15 p.p.m. in ingested foods[7] is a physiological protective mechanism. But concentrations less than 13–15 p.p.m. in foods are also of concern because 5–10% of cadmium ingested by humans is absorbed and retained for many years, chiefly in the kidneys (its biological half life is 16–33 yr)[1]. Consequently, cadmium has been implicated as a possible cause of hypertension[13] through damage to the renal cortex[1].

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Young tomato fruits induced to export carbon by cooling

In phloem translocation systems, sources and sinks are clearly defined· sources are net exporters and sinks net importers of carbon compounds. The overall rate of translocation is largely controlled by ill-defined metabolic processes operating at the source and sink, the transport process itself rarely being limiting[1–3]. An understanding of how this control is exerted is needed before the mechanism of phloem translocation can be understood, and may provide the potential for manipulating the yield of the economically important parts of plants. The direction of net translocation is reversed as a growing leaf matures[3,4], or when previously imported material is redistributed from storage organs such as bulbs[5,6]. In such cases, however, it is difficult to relate causally the physiology or biochemistry of the organ to the change in the direction of translocation. Previous attempts to convert mature leaves from net exporters to net importers have been only partially successful[7–10]. We have now achieved complete reversal of the normal role of a sink, and report here the net export of considerable quantities of carbon from a young, growing tomato fruit.

Table 1 Rates of carbon translocation into (+) or out of (−) tomato fruits at 5 or 25 °C and the concomitant changes in total carbon (Δ carbon) and sucrose (Δ sucrose) during 48 h

Fruit temperature (°C)	Initial carbon content (g)	Δ Carbon (mg per 48 h)	Carbon respired (mg per 48 h)	Carbon translocated (mg per 48 h)	Δ Sucrose (mg per 48 h)
5	1.0−1.3	+ 96.1±15.8	4.5±0.5	+100.6±15.4	+28.3±7.2
25	1.0−1.4	+234.3±10.6	19.1±2.4	+253.4±13.0	− 3.4±7.7
5	1.6−1.9	− 84.5±28.8	5.6±0.5	− 78.9±28.8	+36.8±8.6
25	2.0−2.2	+126.3±16.3	34.5±13.0	+160.8±3.8	− 4.8±4.3

Carbon contents were estimated by a dichromate consumption method[14]. Respiration was measured by the absorption of CO$_2$ in NaOH solution. Fruits were cooled by immersion of the gas exchange chambers in ice–water mixtures, thus maintaining the fruit temperatures at 5±1 °C throughout the experiment. Sucrose contents were determined by autoanalysis as the difference between the total sugars and hexoses in 80% ethanol extracts. The results are expressed as the means ±s e. of two or three fruits

Carbon translocation to or from an individual fruit was measured as the sum of the change in carbon content and the net respiratory loss of carbon during 48 h. Tomato plants were 'stopped' at the 15th leaf, with all fruits but two on the first truss removed. In this twin-fruit system the carbon contents of the two fruits per unit volume were very similar. Thus the initial carbon content of one fruit could be calculated from its volume if the carbon content and volume of the other fruit were measured. At the start of the experiment the volumes of both fruits were measured; one fruit was then removed for analysis of total carbon, while the other was enclosed in an airtight Perspex chamber for measuring its net loss of CO$_2$ during 48 h. The final carbon content of this fruit was determined directly. During the experiment the fruit was maintained at either 5 °C (cooled) or 25 °C (control), with the rest of the plant at 25 °C.

Table 1 shows the results of a typical experiment with data from cooled and control fruits grouped according to size. The fruits were rapidly growing and green, and had reached 25–50% of their final size. The smaller control fruits imported carbon at a greater absolute rate than the larger fruits. The amounts of carbon imported in 48 h were 21% and 8% of the initial carbon contents of the smaller and larger fruits, respectively. Cooling greatly reduced the amount of carbon imported by smaller fruits (to 9% of the initial carbon content), but caused a net loss of carbon from larger fruits (by 5% of the initial carbon content); only 7% of this total loss was due to respiration, and so the balance must have been exported from the fruits. Thus the rate and direction of translocation are related to both fruit size and temperature. Sucrose is the major carbon compound translocated in the phloem in the tomato[11], but in the green fruit as a whole it comprises less than 2.5% of the dry weight. It is interesting, therefore, that although total sucrose content changed little in the control fruit, there were pronounced increases in the cooled fruits. If the low sucrose content of a green fruit is due to intensive conversion of imported sucrose, the increase in sucrose content of the cooled fruits must be due to either a reduced ability to metabolise imported sucrose or to enhanced synthesis of sucrose from other metabolites.

The effect of cooling on the import of carbon by the fruit was also examined by estimating amounts of ^{14}C-leaf assimilate imported during 48 h. The results (Table 2) show that ^{14}C-leaf assimilate was imported by all the cooled fruits, although to a lesser extent than by the controls. Thus, leaf assimilate may move into a cooled fruit even during periods of net carbon export. Furthermore, the percentage increase of ^{14}C-sucrose in the larger cooled fruits (found to be net exporters of carbon) was far greater than in the smaller cooled importing fruits, and about six times that in the controls. The greater percentage increase of ^{14}C-sucrose in the cooled exporters was therefore associated with a high accumulation of sucrose.

Reductions in the accumulation of ^{14}C-leaf assimilates in the roots and the young leaves of sugar beet[12] and the ear of wheat[13] have also been achieved by local cooling. In the

Table 2 Percentage increases in total ^{14}C and ^{14}C-sucrose in tomato fruits at 5 or 25 °C during 48 h

Fruit temperature (°C)	Initial total carbon content (g)	Net carbon translocation	Total ^{14}C (% increase)	^{14}C-sucrose (% increase)
5	1 6–1.9	Export	379±34	405±97
5	1.0–1.3	Import	520±21	184±39
25	1.0–1.4	Import	628±29	70±23

^{14}CO$_2$ (125 μCi) was applied to the leaf above the truss 24 h before the start of the experiment The increase in ^{14}C in the fruit was the difference between its initial and final content of ^{14}C during 48 h. The initial ^{14}C content was estimated by the twin-fruit technique described in the text. The results are expressed as means ± s.e. of two or three fruits light of our findings there may have been net export of carbon from these cooled sinks as in the tomato, although it could not be detected by the techniques used.

Clearly, actively growing, and therefore importing, fruits can be induced, depending on their size, to export considerable quantities of carbon simply by local cooling. This conversion of a net importer to a net exporter is related to reduced import of leaf assimilate and a high accumulation of sucrose in the cooled fruit. Both the rate and direction of carbon translocation in the tomato seem to be related to changes in the sucrose content of the fruit, not merely to the availability of leaf assimilates. Further experiments involved the injection of ^{14}C-sucrose, ^{14}C-glucose or ^{14}C-fructose individually into fruits which were subsequently cooled for 48 h. In all cases autoradiography of peduncle and stem sections showed that the exported ^{14}C-activity was confined to the phloem.

These findings suggest that the status of an organ as a source or sink is determined by the carbohydrate metabolism of that organ, which may be modified by changes in environmental factors, such as temperature. By modifying the chemical conversions of the unloading process in the sink, the activity of that sink can be altered. The observed temperature effect may to a certain extent explain the observation that tomato fruit growth is reduced at low temperatures even when solar radiation is high.

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Circadian activity rhythm influenced by near zero magnetic field

CIRCADIAN periodicity has been observed in many organisms. Motor activity, body temperature, and oxygen consumption are among the functions shown to possess a diurnal rhythm. Many of these rhythms are easily entrained to a natural or artificial cycle of light and darkness. In constant laboratory conditions of light and temperature, rhythms persist, but are slightly longer or slightly shorter than 24 h (ref. 1). The persistence of rhythms in constant conditions together with the evidence from translocation experiments[2], are considered evidence that rhythms are endogenous. Many researchers, among them Pittendrigh[3], Aschoff[4], and Enright[5], adhere to the endogenous rhythm hypothesis.

The possibility that circadian rhythms might be influenced by some geophysical variable such as the diurnal fluctuations in the intensity of the Earth's normal magnetic field, was suggested by Brown[6]. Brown *et al*[6–8] showed that the circadian rhythms of fiddler crabs and other organisms are influenced by small changes in the intensity of the Earth's magnetic field. Wever[9] showed that the circadian activity rhythm of humans may be entrained by an artificial 10 Hz electromagnetic field. Keeton *et al.*[10] demonstrated that the orientation ability of pigeons is influenced by small variations in magnetic field intensity. Elimination of the vertical component of the Earth's field influences the orientation ability of the European robin, *Erithacus rubela*[11].

Since a time sense is important for orientation, and some evidence suggests that birds can be affected by small changes in artificial magnetic field intensity, we studied the effect of these changes on the circadian rhythm of birds, and have found that the circadian activity of the house sparrow (*Passer domesticus*) can be entrained to a cycle of change in the intensity of the Earth's magnetic field.

The characteristics of the circadian activity rhythm in the house sparrow have been well established by Menaker[12] and Eskin[13]. House sparrows entrain especially well to any reasonable light : dark (LD) cycle. Their period length generally becomes slightly longer in conditions of constant darkness.

The birds were captured in the vicinity of Kingston, Rhode Island. Eight adult males and eight females were selected at random. They were housed singly in non-magnetic 0.028-m^3 cages. Two 1.83 m×1.83 m×1.37 m Weber coils were constructed with were identical except that only the experimental coil was activated by a Sorenson power supply. The activated coil produced a field with a total intensity of 0 ± 40 nT within a sphere 0.91 m in diameter within the coil. The total intensity of the Earth's field within the control coil was 0.42 gauss, as measured both when the experimental coil was activated, and when it was not activated. A platform was built for each coil so that the cages could be arranged symmetrically within the sphere of 0 gauss. Eight birds (four males and four females) were placed in each coil. Perch hopping activity was recorded following Eskin's method[13]. The wooden perch was long enough to extend outside the boundary of the coil where it was connected to a microswitch. A barrier was placed parallel to the perch inside the cage, so that the bird would have to hop on the perch to cross the cage. The range of temperature fluctuation was approximately 5 °C. Extraneous noise was masked by a white noise generator which maintained a constant background noise of 84 dB.

For the first 2 weeks all the birds were maintained in an LD 8 : 16 cycle, and in the Earth's normal magnetic field (MF). For the second 2 weeks all the birds were maintained in the LD 8 : 16 cycle. The control birds were kept in the Earth's MF, but the experimental birds were exposed to 8 h of near 0 gauss MF and 16 h of the Earth's MF. The 8 h of near 0 gauss MF were synchronised with the 8 h of light. For the last 4 weeks all the birds were maintained in DD. The controls were kept in the Earth's MF. The experimental birds were kept in the MF cycle as described for the previous 2 weeks. The birds were fed and watered, and the intensity of the MF was measured once or twice a week. The field fluctuated only ±200 nT during the course of the experiment.

The first 2 weeks were used to entrain the birds to the LD 8 : 16 cycle. The second 2 weeks allowed the experimental birds to adjust to the MF cycle. During the last 4 weeks of the experiment, it was expected that the control birds would free run. It was expected that the experimental birds would show a lesser tendency to free run, if they were in fact capable of being influenced by the MF cycle.

Enright's[5] periodogram and analysis of covariance[14] were used to test the null hypothesis that there was no difference in the circadian rhythm of the two groups during the 4 weeks in total darkness.

The event recorder records for the first 4 weeks of the

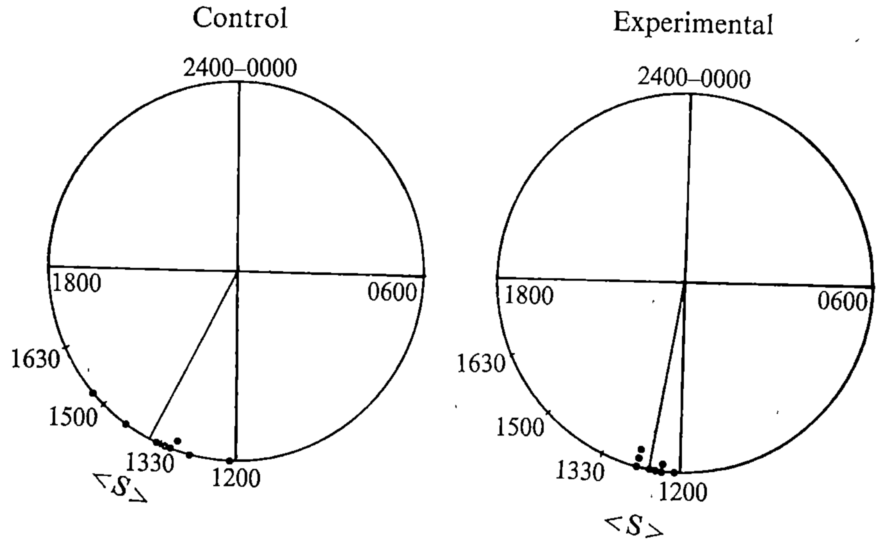

Fig. 1 Mean midpoint of activity in total darkness. This is a circular representation of the times of the midpoints of activity for each bird. 0000 and 2400=360°. 1200=180°. R is a measure of concentration about the mean time (the higher the concentration, the closer R is to 1). S is the mean angular deviation (similar to the standard deviation). The mean time of the midpoint for the control group was 1348 (207°). $R=0.95$. $S=$ 1 h 7 min (16.75°). The mean time of the midpoint for the experimental group was 1242 (190.5°). $R=0.99$ $S=15$ min, (3.75°).

experiment show that both the control birds and the experimental birds entrained strongly to the LD 8 : 16 cycle as expected. The introduction of an 8 : 16 MF cycle during the second 2 weeks had no observable effect on the experimental birds.

During the final 4 weeks of the experiment, the period length of most of the control birds became longer; the onset of activity began later and later each day. This resulted in a large amount of activity from 1800 to 2400.

The activity period of the experimental birds lengthened in most cases. This lengthening was, however, due to the activity beginning earlier and ending later than it had while the light cycle was in operation. The event recorder records show no evidence of a phase shift taking place; the midpoint of activity fluctuated about a mean of 12 noon as it had during the light cycle. The activity between 1800 and 2400 was considerably less than that of the controls.

Enright's periodogram was used to determine the total period length. All the birds showed a marked 24-h period length during the first 4 weeks. The control birds had a period length of approximately 24 h 30 min during the last 2 weeks of the experiment. The experimental birds had a period length of 24 h; however, the relative amplitude of the 24-h period was less marked than in LD 8 : 16. The activity between 1800 and 2400 was significantly greater for the controls than for the experimentals during the 4-week total darkness period (analysis of covariance, $P<0.05$).

Batschelet's[15] method was used to determine the mean times of the midpoint of activity for each bird, both in LD 8 : 16 and in total darkness. The mean times were determined for the control group, and for the experimental group. In LD 8 : 16 the F test showed that there was no significant difference between the two groups. In total darkness the time of midpoint of activity for the experimental group was significantly earlier than for the controls ($P<0.025$) (see Fig. 1).

The results demonstrate that the circadian activity of the house sparrow may be entrained by a cycle of change in the intensity of the vertical component of the Earth's MF. The MF seems to act as a weak Zeitgeber compared with the effect of light. House sparrows, however, show a strong degree of synchronisation with a light cycle.

Enright's periodogram analysis shows that the period length of the experimental birds does not become significantly longer in total darkness, from that of the control birds. The activity period of the experimental birds did not shift significantly out of phase with the previous light period, whereas the activity of the control birds did show a shift.

There were no differences in activity of the controls or experimentals during the first 4 weeks. This is probably because the MF cycle was synchronised with the light cycle, and the 0 MF took the place of light as a potential Zeitgeber only when the light cycle was removed. The experimental birds did not adapt to the MF cycle (in the sense of no longer being influenced by it) during the 6 weeks that the experiment was in operation.

It is possible that the activity of each bird might not be independent of the activity of the other birds. Auditory cues were, however, masked by the white noise generator. Since the cages were placed so that only two (one control bird and one experimental bird) of the birds were able to see each other, visual cues seem an unlikely source of entrainment.

Possibly the ability to be influenced by a near 0 gauss MF is species specific. Or it may influence only such parameters as circadian rhythms and navigation. This might explain why researchers have had little success in demonstrating the effects of intensity changes on human reaction time[16], and other parameters of human behaviour[17]; or in training birds to respond to changes in intensity as a conditioned stimulus[18,19].

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Observations on nudging cells in culture

CELL behaviour has been studied widely in tissue culture, and in many cases individual cells have been shown to inhibit the locomotion of cells with which they make contact[1]. In the embryo, however, cells move mainly in sheets[2] or streams[3-5] and in the latter case their locomotion does not appear to be contact inhibited[6]. The question, then, is how the behaviour of individual cells is coordinated in these organised morphogenetic movements. While studying isolated deep cells of the fish, *Fundulus heteroclitus*, we have made some observations which relate to this question.

Deep cells of blastulae form hemispherical bulges of the cell surface called blebs. As cell locomotion begins during gastrulation some blebs extend to form lobopodia or flatten to form lamellipodia[6]. In films of deep cells blebbing within the embryo it is often difficult to determine whether cells are in contact, and if so, to what extent. Fortunately, however, deep cells isolated from blastulae in culture behave in the same way as cells in the embryo[7].

Blastoderms (stage $11\frac{1}{2}$) were isolated and their deep cells were disaggregated mechanically by flushing through a narrow-bore micropipette. This resulted in a suspension of single cells and small clusters which was placed in a deep watch glass containing a simple culture medium[8]. Most of the cells stuck to the glass substratum within 15 min, although they did not flatten but rather adhered over a small portion of the cell surface as *in vivo* in the blastula. This may have been due to the lesser deformability of cells at this stage[9]. They resumed blebbing during this time. A bulge formed which expanded into a bleb in about 5–8 s. Blebs seemed to be laterally restricted and only involved a small area of the cell surface. Such a bleb was eventually resorbed into the cell and after a variable time another bleb formed almost diametrically opposite the position of the first. The positioning did not seem to be random, in contrast to amphibian gastrula cells[10]. In no instance, in observations of more than 50 cells, has a bleb resorbed and a new bleb formed in the same position.

Most blastula cells in culture initiated new blebs every 30 s. To see if cells in contact influenced each others' blebbing, two attached blastula cells were observed. Both were 40 μm in diameter and had a broad contact between them so that each cell was flattened along the contact. These cells were observed every 15 s for 5 min and 70% of the time they were both blebbing or not blebbing. Many observations of non-contacting deep cells would be required to substantiate a correlation of blebbing between cells in contact. Instead of this, we have taken advantage of the fact that we can stimulate a cell to bleb by nudging or stroking it with a micropipette or smoothed glass probe controlled by a micromanipulator.

Eight individual blastula cells were observed in culture for 2 min, during which one of the cells did not bleb while the other seven initiated a new bleb every 30 s. These cells were then nudged. The cell which had not blebbed did not form a bleb when nudged nor during the next minute. Six cells blebbed within 10 s of being nudged, three of these within 5 s. One of these cells already had a bleb but a new bleb was initiated 10 s after nudging. The remaining cell also had a bleb but did not initiate a new bleb after nudging when observed for 1 min. The bleb which formed on nudging was always diametrically opposite the point where the cell was touched, or almost so. In no case did the bleb form where the cell was touched.

Touching the cell thus seems to stimulate blebbing. This suggests that the increased number of blebbing deep cells *in vivo* as development proceeds during the blastula stage[6] may be due to the increased probability of blebbing cells touching each other. We suggest in addition that this sensitivity to touch *in vitro* also operates when cells move in streams *in vivo*, as in the *Fundulus* germ ring during gastrulation, and that cell movement forward is enhanced by cells bumping into the rear of cells ahead of them. This mechanism would only operate if the cell movement is directional, as in morphogenetic movements. Curtis[11] has pointed out that such shearing of cells against each other would effect viscosity changes in their surfaces, and in appropriate conditions could promote motility.

Amoebae are also stimulated to produce pseudopodia when prodded with a needle, but in this case the protrusion is formed near the site of stimulation. Goldacre[12] has suggested that production of a pseudopod is stimulated by the membrane interacting with the endoplasm of the cell. Although in deep cells there is no evidence for sol–gel transformation, the blebs formed may be analogous to the ectoplasm of an amoeba in that cytoplasm seems to flow into a bleb as it elongates to form a longer protrusion.

To test whether the stimulus for blebbing could be transmitted to another cell, nine doublets of blastula cells adherent to the substratum were studied. One of the two cells was chosen at random to be nudged away from the region of contact. In one doublet, neither the cell nudged nor the other blebbed for 30 s after nudging. In all other doublets both cells blebbed simultaneously; five after 6 s, two after 10 s, and one after 25 s. The blebs formed in the nudged cells in the same position as in single cells. The blebs forming in the attached cells were usually at one side of the region of contact between the cells. This suggests that the surface activity of the cells of the doublet is linked.

As *Fundulus* deep cells have been shown to be electrically coupled[13], we suggest that this might be the mechanism whereby surface activities of cells are coordinated. It is clear, however, that contact with another cell also restricts the activity of an individual cell. Blebs do not form at points of contact and it is possible that the cell surface is not free to flow in these regions (our work in preparation). The question is whether surface activity in groups of cells is coordinated by interaction of the membranes themselves or by messages passing through communicating channels between cells. The same question applies to the local effects on ruffling[14] and contraction[15] on contact with another cell.

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Diminished content of plasma membrane-associated myosin in transformed fibroblasts

MYOSIN, a major contractile element of muscle, has been found in a variety of non-muscle cells such as platelets[1,2], fibroblasts[3,4], brain tissue[5], amoebae[6] and slime mould[7]. It is localised in the plasma membrane[8] as well as in the cytosol[4] of cultured fibroblasts. Although no definite function has been established for non-muscle myosins, they may participate in cellular movement, mitosis, changes in cell shape and related functions. We have examined the protein subunit composition of plasma membranes from normal and transformed fibroblasts with a particular emphasis on determining the content of plasma membrane-associated myosin. We present here evidence that the content of plasma membrane-associated myosin is diminished in transformed fibroblasts. This decrease may in part account for the abnormal shape and loss of contact inhibition of movement of transformed cells[9].

Plasma membranes from normal and transformed rat kidney fibroblasts were prepared by a slight modification of the method of Hochstadt *et al.*[10] in which subcellular fraction is carried out using hypertonic sucrose and its polymer Ficoll. Examination of the membrane fraction by electron microscopy showed that about 90% of the structures were closed membrane vesicles and less than 10% were mitochondria. Marker enzyme studies using adenylate cyclase and Na^+, K^+–ATPase confirmed that the membrane fraction contained purified plasma membrane, with enrichment ranging up to 10 times greater than homogenates. Furthermore, the contamination of the membrane fraction with endoplasmic reticulum, as assessed by the specific activity of the marker enzyme NADH dehydrogenase[10], was comparable between normal and transformed cell lines.

Figure 1 shows a typical electrophoretogram of homogenates and plasma membranes from normal and transformed cells, in which equal amounts of protein were applied. In this experiment, the protein subunit patterns from the normal and transformed whole cell homogenates were quite similar. On the other hand, when the protein subunit patterns of plasma membranes of transformed fibroblasts were compared with those of normal fibroblasts, a marked decrease was observed in a protein band with a molecular weight of 200,000. This band corresponds to the heavy chain of fibroblast myosin as indicated by the arrows in Fig. 1. As previously noted the heavy chain of fibroblast myosin migrates slightly faster than the heavy chain of the skeletal muscle myosin standard used[3,4]. The band in the plasma membrane preparations migrating slightly slower than myosin probably represents the myosin-associated protein of molecular weight 210,000, recently described by Olden *et al.*[11], and is immunologically unrelated to myosin (K.O., M. C. Willingham and I.P., manuscript in preparation). The quantitative measurement of the myosin band by densitometry using purified myosins from L cells and rabbit muscles as standard calibration proteins indicated that myosin was decreased by 60–75% in transformed membrane preparations. Table 1 summarises the average values of membrane-associated protein of molecular weight 200,000 from normal and transformed cells when three different plasma membrane preparations were used.

Table 1 Content of membrane-associated 200,000-molecular weight protein

Cell lines	μg myosin–mg membrane protein	%
NRK	42	100
HNRK	19	45
KNRK	13	31
MNRK	11	26
SNRK	16	38

Quantitative determination was performed using Gilford model 400 spectrophotometer equipped with a densitometric apparatus and slab electrophoretic gels with three different preparations of normal and transformed membranes. The content of the protein in the membranes was computed by dividing the area of protein in the myosin band (determined by densitometry) by the total area of all the stained bands, and the results are expressed as μg per mg of total membrane protein.

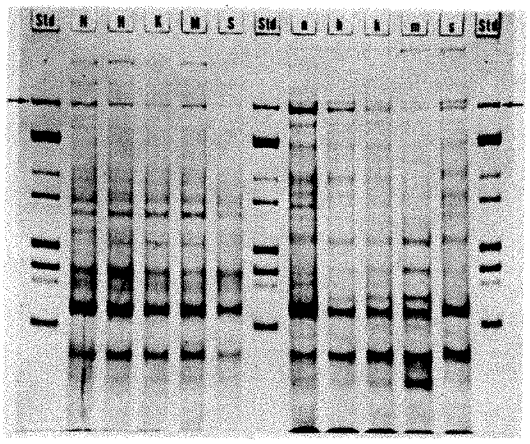

Fig. 1 Normal rat kidney fibroblasts (NRK), and NRK cells transformed with Harvey (HNRK), Kirsten (KNRK), Moloney (MNRK) and Schmidt-Ruppin (SNRK) viruses were grown to confluency in 32-ounce bottles using Dulbecco–Vogt medium supplemented with 10% calf serum. Each bottle was washed twice with 100 ml of 10 mM phosphate buffer, *p*H 7.5, containing 0.15 M NaCl (PBS) and once with 100 ml of 10 mM Tris–HCl, *p*H 8.0, containing 0.5 M sucrose, 0.1 mM dithiothreitol and 0.1 mM EDTA (Tris–sucrose buffer). The cells were scraped into 10 ml of buffer. The plasma membrane fraction was isolated at 1–4 °C by the following modification of the method of Hochstadt *et al.*[10]. The mixture from each bottle was homogenised with 10 strokes of a Potter homogeniser and unbroken cells were further homogenised with five strokes of a Dounce homogeniser. A sample was taken from each homogenate for electrophoresis. Then 5 μg of DNase and magnesium sulphate at final concentration of 0.1 mM were added to the residual homogenates. The nuclear fraction was removed by centrifugation at 1,000*g* for 5 min. The supernatants were further centrifuged in a SW30 rotor at 4 °C at 20,000*g* for 15 min. Each pellet thus obtained was suspended in 5 ml of Tris–sucrose buffer and the suspension layered on 10% Ficoll dissolved in Tris–sucrose buffer. Centrifugation using a Beckman SW40 rotor was performed at 100,000*g* for 2 h. The interface layer was collected and washed with 10 ml of Tris–sucrose buffer containing 0.1 mM magnesium sulphate, followed by centrifugation at 20,000*g* for 15 min. The pellet was suspended in a small amount of Tris–sucrose buffer containing 0.1 mM magnesium sulphate and designated as the plasma membrane fractions. Then, each membrane fraction or whole cell homogenate from normal and transformed cells was dissolved in 2% SDS containing 100 mM dithiothreitol, 10 mM potassium phosphate buffer, *p*H 7.0, 29% glycerol and 0.03% bromophenol blue. Samples were heated at 100 °C for 3 min. Approximately 25 μg of each sample was applied on a SDS slab 5% polyacrylamide gel (1 mm width) and electrophoresis was performed according to the method of Studier[12]. Protein staining was performed using 0.25% Coomassie blue in 50% trichloroacetic acid. Excess dye was removed by incubating the gel with 7% acetic acid. Std represents the gel electrophoretic pattern of the following molecular weight standard proteins: skeletal muscle myosin (200,000, see arrow), RNA polymerase (160,000 and 150,000) β-galactosidase (130,000), phosphorylase *a* (95,000), bovine serum albumin (68,000) and ovalbumin (43,000). On the left are the gel patterns of whole cell homogenate from NRK (N), HNRK (H), KNRK (K), MNRK (M) and SNRK (S), and on the right are the gel patterns of the membrane fractions from NRK (n), HNRK (h), KNRK (k), MNRK (m) and SNRK (s). The lower band of the doublet in the 200,000-molecular weight range of the membrane fraction is myosin. The upper band correlates to a myosin-associated protein[11].

Table 2 EDTA–ATPase activity in whole cell homogenates and membrane preparations from normal and transformed cells

Cell lines	EDTA–ATPase Homogenate (nmol min^{-1} mg^{-1})	%	Membrane (nmol min^{-1} mg^{-1})	%
NRK	0.88	100	1.41	100
HNRK	0.63	72	0.57	40
KNRK	0.67	76	0.47	33
MNRK	0.68	77	0.52	37
SNRK	0.73	83	0.58	41

EDTA–ATPase activity was determined by the following modification of the method of Ostlund *et al.*[4]. The reaction mixture containing 25 mM 2-*N*-morpholinoethane sulphonic acid buffer, *p*H 7.0, 0.2 mM γ-^{32}P-ATP (50 c.p.m. pmol^{-1}), 0.6 M KCl, 2 mM EDTA, 1 mM dithiothreitol, 1 mM ouabain and the enzyme sample in a final volume of 0.1 ml. Incubation was performed at 37 °C for 20 min. The reaction was stopped by adding 0.5 ml of 20% trichloroacetic acid and the precipitate formed was removed by centrifugation. To 3 ml of supernatant were added 0.1 ml each of 5% ammonium molybdate in 5 N sulphuric acid and 30 mM silicotungstic acid. The molybdate–phosphate complex was extracted with 2 ml of 50% isobutyl alcohol and 50% benzene[13], and sample of the organic phase was used for determining radioactivity. This assay was linear with respect to time and amount of the enzyme sample in the conditions used.

To identify the 200,000-molecular weight protein band as the heavy chain of myosin, the sodium dodecyl sulphate (SDS) gels were stained as before (K.O. and K. M Yamada, manuscript in preparation), using goat antimyosin λ-globulin, rabbit antigoat λ-globulin conjugated with horseradish peroxidase, 3,3′-diaminobenzadine and hydrogen peroxide. A peroxidase-positive band was observed in the position of the 200,000-molecular weight protein, indicating that the protein that was decreased in the transformed cell membranes was immunochemically very similar to fibroblast myosin.

To confirm that the content of membrane-associated myosin was diminished in transformed fibroblasts, we measured the characteristic EDTA–ATPase activity (myosin ATPase)[3] using normal and transformed cell membranes as well as whole cell homogenates Table 2 shows the results of such experiments. The levels of EDTA–ATPase activity in the transformed cell plasma membranes were always 20–40% of that in the normal cell membranes, whereas the EDTA–ATPase activities of the whole cell homogenates from the transformed cells were always 70–95% of that in the normal cell homogenate. Another point to be noted in Table 2 is that the concentration of myosin in the plasma membrane fraction is only slightly greater than its concentration in the whole cell homogenate. Thus myosin is not concentrated in the plasma membrane fraction.

Table 3 shows the effect of antimyosin λ-globulin on EDTA–ATPase activity of purified fibroblast myosin and a fibroblast membrane preparation. In the conditions used approximately 60% of EDTA–ATPase activity was inhibited with antimyosin λ-globulin when both purified myosin and fibroblast membranes were used. Control globulin preparations inhibited EDTA–ATPase activity by less than 15%, indicating that the bulk of EDTA–ATPase activity measured is derived from myosin ATPase activity. Since the results obtained from the quantitative densitometric analyses of membrane-associated myosin (Table 1) agree with the data showing the decreased levels of EDTA–ATPase activity (Table 2), we conclude that membrane-associated myosin is diminished in these transformed rat kidney fibroblasts.

Table 3 Effect of antimyosin γ-globulin on EDTA–ATPase activity

Enzyme source	EDTA–ATPase (nmol min^{-1} mg^{-1})	Inhibition (%)
Myosin from L cells	6.0	0
+antimyosin γ-globulin (100 μg)	2.1	65
+antimyosin γ-globulin (200 μg)	2.2	63
Membrane preparation from NRK	1.48	0
+antimyosin γ-globulin (100 μg)	0.54	64
+antimyosin γ-globulin (200 μg)	0.58	61
+antirabbit γ-globulin (200 μg)	1.26	15
+human γ-globulin (200 μg)	1.36	8

The assays were performed as described in the legend to Table 2. Purified L-cell (15 μg) myosin and 100 μg of NRK membrane protein were used.

Recent reports from several laboratories[14–16] have indicated that the content of 200,000-molecular weight protein subunit is decreased in plasma membranes from transformed chick embryo fibroblasts. Although the 200,000-molecular weight protein was not identified, it seems likely that the protein subunit that is diminished in chick cell membranes is also the heavy chain of myosin, if the present and previous experimental results[4,8] from our laboratory are compared with these reports[14–16]. It needs to be emphasised that myosin is distinct from a higher molecular weight glycoprotein (220,000) recently isolated from the surface of chick embryo cells[17] with a content markedly decreased after transformation or trypsin treatment.

Treatment of some transformed cells with dibutyryl cyclic AMP induces flattening and elongation, morphological changes which make the transformed cells resemble in some respects their normal parent cells (see review in ref. 18). Therefore we attempted to determine the content of membrane-associated myosin as well as EDTA–ATPase activity in plasma membranes from a SV40-transformed BALB/3T3 cell line that was cultured in the presence and absence of 1.5 mM dibutyryl cyclic AMP and 0.5 mM methyl isobutyl xanthine, a phosphodiesterase inhibitor. This cell rather than a transformed NRK cell was chosen because it has a very striking morphological response to the nucleotide The results indicated the EDTA–ATPase activity and the content of membrane-associated myosin was increased by less than 30% when the cells were treated with dibutyryl cyclic AMP. Thus the amount of myosin associated with the plasma membrane in this cell line does not seem to be affected by cyclic AMP. The mechanism responsible for the low content of myosin in plasma membranes of these transformed cells is under investigation.

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Two mechanisms for spontaneous recovery from depolarising drugs in rat muscle

THE action of depolarising drugs on the endplate of skeletal muscle is difficult to interpret because the depolarisation is often transient and followed by spontaneous recovery during the continued presence of the drug, and because of differences between species. In frog muscle, depolarising drugs produce an initial increase in conductance at the endplate, followed by a decline in the conductance as 'desensitisation' develops[1-3]. In cat muscle, depolarisation is maintained while the drug is applied[4]. Rat muscle has long been considered anomalous in that the depolarisation is transient and limited[5]. We now report an unexpected finding for rat diaphragm, in which the recovery is attributed to the action of an electrogenic sodium pump[6], stimulated by the entry of sodium.

Figure 1 shows the action of carbachol (100 μM) recorded with an internal electrode with tetrodotoxin (0.1 μM) in the outside bath to prevent action potentials. The recovery of the membrane potential in the presence of the drug might be due to closure of the ion channels after the initial action of the drug, or to another process. There are methods by which the entry of labelled sodium in the junctional region can be studied directly in diaphragm muscle[7], and dose-response curves have been obtained[8]. Figure 2a shows the uptake of ^{24}Na in a diaphragm exposed for 15 s to a solution containing ^{24}Na and carbachol (100 μM) followed by washing for 6 min. Compared with the control (Fig. 2b), there was an extra uptake of ^{24}Na in the junctional region in the presence of the depolarising drug. The effect was similar (Fig. 2c) when the diaphragm was pretreated with carbachol (100 μM) for 30 min. These results (and others to be reported elsewhere) are consistent with the view that the ion channels continue to open while the drug is in contact with the tissue, and this differs markedly from the situation in frog muscle.

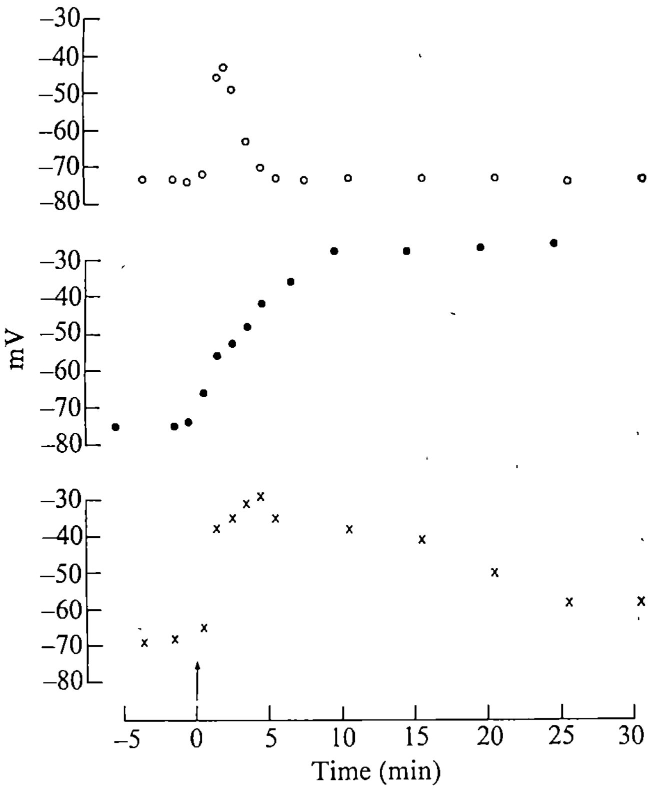

Fig. 1 ○, Effect of 100 μM carbachol at endplate, with internal recording[19]: muscle vertical, arranged for continuous register and rapid changeover, at 38 °C The solution contained 5 mM potassium and 0.1 μM tetrodotoxin. The ordinate gives membrane potential, with depolarisation upwards The abscissa gives time after the solution was changed. Miniature endplate potentials disappeared when carbachol was applied. ●, Effects of 100 μM carbachol plus 100 μM ouabain. The reading after 25–60 min with ouabain plus carbachol 100 μM was -31 mV (median of six estimations). ×, Effect of 3 mM decamethonium plus 100 μM ouabain: final reading, compared with initial resting potential, showed depolarisation of 9 mV (median of nine estimations)

The recovery of the resting potential could be attributable to an electrogenic sodium pump, and in this case the process would be affected by certain enzyme inhibitors. When ouabain[9] and carbachol (100 μM) were added concomitantly (Fig. 1) there was a profound depolarisation and little evidence of recovery. In rat diaphragm, ouabain alone (100 μM at 38 °C) produced a depolarisation of 9 mV (median of 10 estimations, range 6–17 mV) by sampling methods used before[10]. This is a greater effect than that found in frog muscle at room temperature with strophanthidin[11].

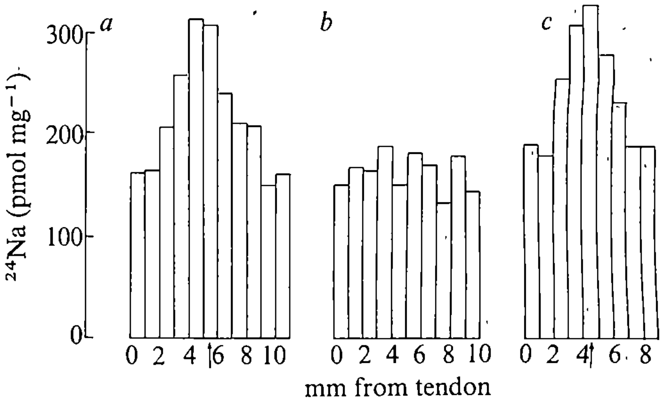

Fig. 2 a, Effect of carbachol on influx of labelled sodium in rat diaphragm in the presence of 0.1 μM tetrodotoxin. The diaphragm was exposed for 15 s to solution containing carbachol (100 μM) plus ^{24}Na, (1 mCi in 10 ml), then washed for 6 min in inactive saline, frozen, sliced and counted[7,20]. Ordinate gives ^{24}Na (pmol mg^{-1}); abscissa is distance from tendon (mm). The arrow indicates the strip of muscle which contained the band of endplates[20]. b, Control without carbachol The uptake in a, above the level assigned to the ends of the muscle, gives the extra sodium which entered in the presence of carbachol and which remained after washing for 6 min: extrapolation to zero time is needed to give the uptake at the end of the exposure. c, Uptake in a diaphragm which had been incubated for 30 min with 100 μM carbachol and then treated as in a.

Figure 1 shows that the membrane potential returned, in the presence of carbachol, to a level close to the initial reading, and in nine experiments the difference ranged from −9 mV to +4 mV. It is not clear how the voltage could be regulated to give recovery to the previous value, and in other situations, with a higher initial resting potential, the recovery from depolarising drugs was incomplete in rat muscle[5].

Decamethonium (100 μM) in the presence of ouabain had an effect similar to that shown in Fig. 1 (closed circles). But with large concentrations of decamethonium the situation was different Figure 3a shows the uptake of ^{24}Na in a muscle exposed for 15 s to decamethonium (3 mM) and washed for 6 min. There was an extra uptake in the junctional region compared with the value at the ends of the fibres, and this initial entry was comparable with the effect shown in Fig 2a. Pretreatment with decamethonium (3 mM) for 30 min (Fig. 3b) gave little or no indication of an extra uptake of sodium in the junctional region, and we infer that after 30 min at this concentration the channels for sodium had mostly been closed. When a solution containing ouabain (100 μM) and decamethonium (3 mM) was applied, there was an initial depolarisation (Fig. 1, crosses) followed by marked spontaneous recovery.

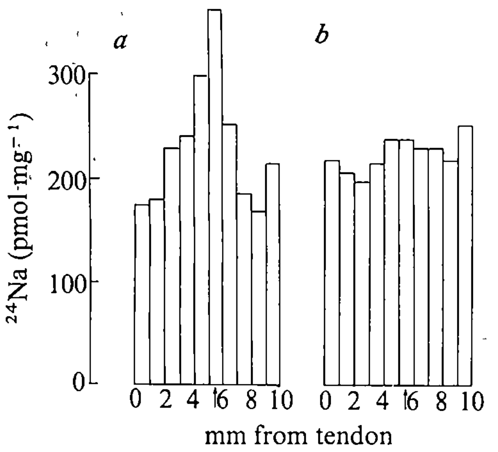

Fig. 3 Effect of 3 mM decamethonium on entry of ^{24}Na: exposure for 15 s, followed by washing for 6 min and treatment as in Fig. 2. The arrow shows the slice of muscle which contained the band of endplates. In *b* the muscle was incubated for 30 min with 3 mM decamethonium and then treated as in *a*.

In rat muscle there seems to be at least two mechanisms for the recovery of the resting potential. The first is an ouabain-sensitive process[12] in which sodium channels continue to open under the influence of the drug. This may explain why labelled decamethonium, which enters at the endplate region[13], accumulates steadily for many hours[14] in spite of desensitisation. The second process is seen with high concentrations of decamethonium and is largely unaffected by ouabain, apparently involving closure of sodium channels. The permeability to decamethonium falls in the mM range in certain conditions[15], and this could be due to channel block as postulated for frog muscle[3]. The alternative explanation in terms of receptor mechanisms is that the pharmacological receptors can be transformed to a desensitised state[16–18] in which ion channels become secondarily closed.

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Psychoactive drug effects on a system which generates cyclic AMP in brain

RECENTLY a direct correlation has been reported between spontaneous motor activity, tyrosine hydroxylase activity and the responsiveness of the noradrenaline sensitive cyclic AMP-generating system in rat brain[1,2]. These observations may indicate that the turnover and/or level of noradrenaline in the brain is important in determining the sensitivity of the cyclic AMP-generating system to noradrenaline. Indeed, it has been shown that depletion of brain noradrenaline by the intraventricular injection of 6-hydroxydopamine results in supersensitivity of the system to noradrenaline[3,4]. Furthermore treatment with reserpine, a drug known to cause depressive reactions in man, has been reported to increase the sensitivity of the adenylate cyclase of rat brain to noradrenaline[5] and several antidepressant drugs have been shown to have the opposite effect in limbic forebrain[6]. By contrast, we now present evidence that the chronic treatment of rats with both an antidepressant, imipramine, and a depressant, chlorpromazine, reduces the sensitivity of the cyclic AMP-generating system to noradrenaline.

Male Sprague–Dawley rats (initial weight 170–180 g) were used in this study. Treatment was carried out once daily. Rats were given 20 mg kg^{-1} of either chlorpromazine hydrochloride or imipramine hydrochloride by an oesophagal tube. Control rats received a corresponding volume of water by the same route. Experiments were always performed 24 h after the last drug application. After specified intervals of treatment rats were decapitated and cerebral cortical slices of 260 μm were prepared as rapidly as possible. Procedures for the preparation, incubation and fixation of tissue have been described in detail[7,8]. Cyclic AMP was determined by the method of Gilman[9] after purification on Dowex 1×8 columns, each sample being analysed at three different concentrations. All values were corrected for recovery and cyclic AMP values were calculated as pmol mg^{-1} protein. Statistical comparisons of results were made using Student's *t* test.

No change of the noradrenaline sensitive cyclic AMP formation was observed in control rats treated for 1 to 21 d with water, therefore all values obtained from these animals were combined (Table 1). When rats were treated with either chlorpromazine or imipramine no significant alteration of the noradrenaline elicited accumulation of cyclic AMP was found after 1 and 3 d of drug administration. After 6 d of daily treatment with drugs, however, a reduced sensitivity developed to noradrenaline and cyclic AMP levels were about 40% lower than controls. This reduced sensitivity of the cyclic AMP-generating system did not change during prolonged drug administration for more than two weeks, when cyclic AMP levels only reached 60% of respective controls after incubation with noradrenaline. There was no difference between animals treated with imipramine or chlorpromazine. The changes in the cyclic AMP system appeared after the same period of pretreatment with either drug and were quantitatively undiscernible in spite of the pronounced differences in their therapeutic effects.

The antidepressant imipramine has been shown to inhibit effectively the uptake of noradrenaline and other biogenic amines into peripheral and central monoaminergic neurones. Imipramine may also interfere with the control of noradrenaline release by blocking presynaptic α-adrenoceptors[10]. Such potentiation of catecholamines at adrenergic receptor sites is generally assumed to be involved in the mechanism of action of imipramine. If this is so then the data presented here indicate that enhanced stimulation of postsynaptic receptors results in the development of subsensitivity to the neurohormone The therapeutic action of antidepressant drugs occurs only after chronic treatment, so it is tempting to speculate that the decrease of receptor function is connected with the drugs' therapeutic action[6].

The mechanism of action of chlorpromazine has been discussed mostly in terms of a blockade of central dopaminergic receptors which is apparently common to nearly all antipsychotic drugs[11,12]. Therefore, it was surprising to find identical results with chlorpromazine pretreatment on the noradrenaline sensitive adenylate cyclase of

Table 1 Effect of imipramine and chlorpromazine treatment on noradrenaline stimulated formation of cyclic AMP in rat brain cortex

Kind of treatment	Duration of treatment (d)	Cyclic AMP (pmol per mg protein ± s e.m.)	
		Additions	
		None	Noradrenaline, 100 µM
Water	1–21	13.6 ± 0.8 (35)	54.6 ± 3 (35)
Chlorpromazine (20 mg kg^{-1})	1	14.0 ± 1.8 (5)	43.8 ± 2.9 (5)
Imipramine (20 mg kg^{-1})	1	13.8 ± 3.1 (5)	48.6 ± 5.2 (5)
Chlorpromazine	3	18.8 ± 6.7 (5)	50.0 ± 7.9 (5)
Imipramine	3	14.6 ± 1.4 (5)	41.0 ± 7.2 (5)
Chlorpromazine	6	10.6 ± 2.2 (5)	32.4 ± 4 5 (5)*
Imipramine	6	11.0 ± 1.5 (5)	27.0 ± 4.0 (5)‡
Chlorpromazine	10	10.8 ± 2 5 (5)	33.8 ± 4.1 (5)*
Imipramine	10	10.0 ± 0.5 (5)*	32.6 ± 5.5 (5)*
Chlorpromazine	15	18.4 ± 2.0 (5)	33.2 ± 6.5 (5)*
Imipramine	15	15.3 ± 1.8 (8)	34.5 ± 4.4 (8)*
Chlorpromazine	21	16.3 ± 2.8 (8)	35.3 ± 2.3 (7)*
Imipramine	21	15.2 ± 3.1 (5)	31.8 ± 5.8 (5)†

Last drug administration was always 24 h before the experiments. Numbers in brackets refer to individual experiments, not multiple determinations. $^*P<0.025$, $^\dagger P<0.015$, $^\ddagger P<0.005$.

rat cerebral cortical slices. Chlorpromazine has, however, been shown to increase the stimulation-induced noradrenaline overflow from field stimulated rat brain slices[13,14], resulting in an enhanced availability of catecholamine at postsynaptic receptor sites, although in some systems it is less active than imipramine in blocking noradrenaline reuptake. The fact that chlorpromazine also blocks α-adrenoceptors, possibly presynaptically located, may also contribute to the enhanced presence of catecholamines at the synaptic cleft[13,15]. This may then trigger the development of subsensitivity of adrenergic receptors to noradrenaline as measured in this study by the formation of cyclic AMP. Any subsensitivity due to a postsynaptic block of α-receptors might be expected to occur after the first dose of chlorpromazine or imipramine. Because of the different therapeutic actions of imipramine and chlorpromazine the significance of the reduced sensitivity of the cyclic AMP-generating system to noradrenaline as a possible common feature of antidepressants requires re-evaluation. It must, however, be remembered that a drug may have more than one action and the fact that chlorpromazine has been shown to have an effect previously only associated with antidepressants does not completely invalidate such an association. Indeed the value of chlorpromazine in conditions like schizophrenia could well depend on its potential antidepressant properties to counter marked depression.

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Substantia nigra of the rat contains a dopamine sensitive adenylate cyclase

A LARGE body of evidence exists to support the hypothesis that most antipsychotic drugs exert their specific actions by antagonising the effects of dopamine at postsynaptic receptors within the central nervous system[1–3]. More recent evidence suggests that postsynaptic dopamine receptors may occur not only at sites of axon terminals, but also within the region of the cell bodies which give rise to the ascending dopaminergic projections. Iontophoretic application of dopamine to dopamine-containing neurone cell bodies in the zona compacta of the substantia nigra, inhibits firing of these cells[4]. Fluorescent histochemical examination of the substantia nigra with the glyoxylic acid technique has shown that dendrites arising from cell bodies of the zona compacta contain intensely fluorescent varicosities, and that these occur both in compacta and reticulata regions of the substantia nigra[5]. These varicosities may form the anatomical basis for a mechanism of self-inhibition whereby dopamine released from dendritic varicosities on stimulation subsequently inhibits the firing of the dopamine neurones themselves[6]. Direct support for the role of dendrites in the release of dopamine has been obtained by showing that slices of substantia nigra labelled by exposure to ^{3}H-dopamine will release labelled dopamine when depolarised by high potassium concentrations[7]. We set out to explore further the hypothesis of self-inhibitory control by looking for biochemical evidence of a dopamine response in this region of the brain. Homogenates of tissues containing dopaminergic axon terminals respond to low concentrations of dopamine by increased production of cyclic AMP[8–12]. We now provide evidence that a similar dopamine sensitive adenylate cyclase is present in the rat substantia nigra, and that it is potently antagonised by antipsychotic drugs.

Using homogenates of tissue from substantia nigra, dissected from fresh sections of chilled tissue (Fig. 1), we found that low concentrations of dopamine were able to stimulate the production of cyclic AMP, half-maximal stimulation being obtained at approximately 2 µM dopamine (Fig. 2). (−)-Noradrenaline was about five times less potent than dopamine giving half-maximal stimulation at approximately 10 µM. Dose–response curves for a number of related compounds were also obtained in this system, and the results are summarised in Table 1. Both the rigid dopamine analogue 2-amino-6,7,-dihydroxy-(1,2,3,4)-tetrahydronaphthalene (ADTN) and epinine (*N*-methyl dopamine), gave dose–response curves similar to dopamine. These results are remarkably similar to those obtained in an

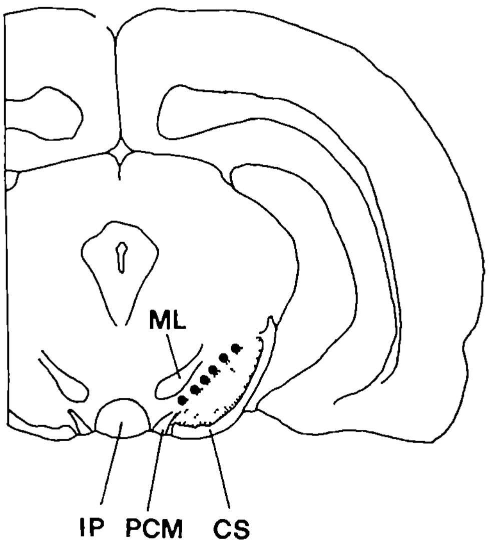

Fig. 1 Diagram of a representative section used for dissection of the substantia nigra. The coordinates for this section are A1760 μM according to the Konig and Klippel atlas[18]. Approximate position of dopamine cell bodies of the zona compacta are shown by the black dots[19]. Zona reticulata is represented by stippled area. Sprague–Dawley rats weighing 180–250 g were used. After decapitation, the brains were rapidly removed and placed on ice. The brain was prepared for sectioning on a McIlwain tissue chopper in a 4 °C cold room in the following way. A horizontal cut was made through the brain removing the dorsal section of the cerebral cortex and including the dorsal portions of the basal ganglia The brain was then placed with its cut dorsal surface face down on the stage of the tissue chopper with a piece of one-sided sticky tape on its surface, and a layer of rapid bonding glue on its upper surface. The brain was aligned to the knife and left for 10 min, its surface moistened by saline soaked filter paper. Sections 300 μm thick were then cut through the whole substantia nigra, using the manual mechanism on the tissue chopper, and the sections transferred to ice cold microscope slides. The sections were examined under a dissecting microscope and the nigra dissected. The mean wet weight of the whole substantia nigra from both sides of the brain was 7.3±0.3 mg ($n = 14$). This method was adapted from that reported by Ben-Ari and Zigmond[20]. The tissue removed for adenylate cyclase assay was bounded by, but excluded, the medial lemniscus superiorly, pendunculus corporis mamillaris medially and the corticospinal fibres inferolaterally. ML, medial lemniscus; IP, interpeduncular nucleus; PCM, pedunculus corporis mamillaris; CS, corticospinal tract.

earlier series of experiments performed in our laboratory on the dopamine sensitive adenylate cyclase of rat striatum[13]. Furthermore, like the striatal enzyme there was a strict requirement for a catechol moiety for agonist activity, while hydroxylation at the β-position reduced activity.

Table 2 summarises the results obtained with a spectrum of antipsychotic drugs. The most potent inhibitor of the dopamine stimulated enzyme was α-flupenthixol, followed in order of potency by chlorpromazine>haloperidol> clozapine=pimozide. The order of potency thus followed that seen for inhibition of the dopamine sensitive adenylate cyclase of the rat striatum. Comparison of the K_i values however shows that the series as a whole seems to be somewhat more potent in the substantia nigra as compared with the striatum

When β-flupenthixol and promazine were tested at a single concentration (1 μM) against the dopamine-stimulated adenylate cyclase, both drugs showed only weak antagonism. Promazine is known to possess only weak antipsychotic activity[14] and β-flupenthixol is virtually inactive as a neuroleptic compound[15].

These results provide direct biochemical evidence that dopamine may function as a neurotransmitter within the substantia nigra. Furthermore, the agonist characteristics of the dopamine sensitive adenylate cyclase in this brain region are virtually identical to those seen in the rat corpus striatum where a neurotransmitter role for dopamine is firmly established[22]. Our results, however, do not enable us to say whether the dopamine receptor is located on the dopamine neurone itself, or on a postsynaptic element Thus, as well as acting on an "autoreceptor", dopamine could act by altering the firing rate of an interneurone, or by modulating the release of other neurotransmitters from presynaptic terminals located on dendrites of dopaminergic cells. Both γ-aminobutyric acid[23] and substance P (ref 24) are present in high concentrations in the substantia nigra and could be involved in such a mechanism.

Whatever the site of action of dopamine, however, the results provide direct support for the hypothesis of self inhibition in a dopaminergic nucleus and further suggest

Table 1 Effect of dopamine and dopamine analogues on the stimulation of cyclic AMP production in homogenates of rat substantia nigra

Agonists	EC_{50}* (M)		Maximal stimulation (% of dopamine response)	
	S. nigra	Striatum†	S. nigra	Striatum†
Dopamine	2.3×10^{-6} M	2.0×10^{-6} M	100	100
ADTN‡	1.5×10^{-6} M	4.0×10^{-6} M	93	115
Epinine	2.8×10^{-6} M	1.5×10^{-6} M	107	100
(−)-Noradrenaline	1.2×10^{-5} M	4.0×10^{-5} M	81	97

(±)-Isoprenaline, *p*-tyramine, (+)-amphetamine, 5-hydroxytryptamine, were all inactive at 1×10^{-4} M.

*EC_{50} refers to the concentration required to give half maximal stimulation.

†Values quoted for striatum are taken from Miller *et al.*[13].

‡2-amino-6,7-dihydroxy-(1,2,3,4)-tetrahydronaphthalene. Basal level of cyclic AMP production was 3.24±0.23 pmol per mg wet weight per min and stimulated level (5×10^{-5} M dopamine) was 4.74±0.11 pmol per mg wet weight per min (mean ± s.e.m for 4 experiments).

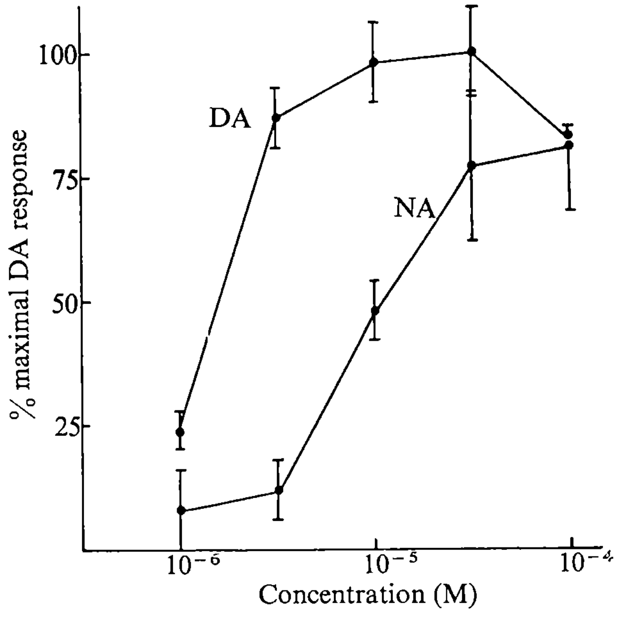

Fig. 2 Effect of dopamine (DA) and (−)-noradrenaline (NA) on stimulation of cyclic AMP production in homogenates of substantia nigra. The homogenisation and incubation procedures were essentially as described by Miller, Horn, Iversen and Pinder[13]. The procedure was, however, scaled down so that single estimations could be performed on 0.4 mg wet weight of tissue. 50-μl aliquots of the final supernatant were assayed for cyclic AMP content by the method of Brown et al [21]. The levels of cyclic AMP increased from a basal level of 2.82±0.16 pmol per mg wet weight per min to 4.33±0.27 pmol per mg wet weight per min (mean value ± s.e.m., $n = 5$) in the presence of a maximally stimulating concentration of DA (50 μM). The experiments with NA were done by comparison with a maximally stimulating concentration of DA and the effect of 50 μM DA normalised to 100%. Results are means ± s.e.m. of 5 separate incubations.

Table 2 Effect of neuroleptic compounds on the dopamine stimulated adenylate cyclase in rat substantia nigra homogenates

Antagonist	IC_{50} (M)	K_i (M)	K_i value for rat striatum (M)
α–Flupenthixol	1.1×10^{-8}	4.8×10^{-10}	1.0×10^{-9}
Chlorpromazine	3.2×10^{-8}	1.4×10^{-9}	4.8×10^{-8}
Haloperidol	1.2×10^{-7}	5.3×10^{-9}	9.1×10^{-8}
Clozapine	4.0×10^{-7}	1.7×10^{-8}	1.7×10^{-7}
Pimozide	4.1×10^{-7}	1.8×10^{-8}	1.4×10^{-7}

Dose–response curves for the antagonists were obtained in the presence of maximally stimulating concentration of dopamine (50 μM). The neuroleptics were added immediately before the addition of dopamine. The IC_{50} refers to the concentration of antagonist required for 50% inhibition of the increase in cyclic AMP resulting from addition of 50 μM dopamine. K_i values for each drug were calculated according to the equation $IC_{50} = K_i\ (1+S/K_m)$ where S refers to the concentration of dopamine and K_m the concentration of dopamine required for half maximal stimulation of adenylate cyclase activity. Competitive inhibition has been assumed for all compounds tested (see refs 16 and 17). Samples testing for inhibition of basal activity by drug (at a concentration of 10^{-4} M) were run with all dose–response curves. Basal level of cyclic AMP production was 2.92±0.12 pmol cyclic AMP per mg wet weight per min and stimulated level (5×10^{-5} M dopamine) was 4.59±0.15 pmol cyclic AMP per mg wet weight per min (mean±s.e.m. for 5 experiments). K_i values for rat striatum are taken from Miller, Horn and Iversen[16].

that antipsychotic drugs may exert at least part of their action by interfering with this mechanism of control.

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Direct localisation of β-adrenoceptor sites in rat cerebellum by a new fluorescent analogue of propranolol

NORADRENALINE (NA) is a putative neurotransmitter, possibly involved in mediation of information transfer across synapses in the mammalian central nervous system (CNS)[1], whose postsynaptic effect is exerted, in part, by stimulation of β-adrenoceptors coupled to adenylate cyclase[2]. Although the rat cerebellum is known to contain NA[3], and an afferent noradrenergic pathway has been found[4–7], little information is available concerning the noradrenergic synapses there. Indirect evidence such as increase in cyclic AMP content and inhibition of spontaneous electrical activity in rat Purkinje cells[8–10] (reduced by β-adrenoceptor blocking[8]), suggests, however, that such synapses are present and that the postsynaptic NA receptors are of the β type. β-adrenoceptors were identified in homogenates of whole rat cerebellum by a binding assay which used the β-adrenergic antagonist ^{3}H-(−)-alprenolol[11]. We have now developed a direct *in vivo* method, using 9-amino-acridin-propranolol (9-AAP), a potent fluorescent β-adrenergic blocker, as a fluorescent probe, in an attempt to detect β-adrenergic receptors in rat cerebellum.

9-AAP (D. Atlas and A. Levitzki, unpublished) is a fluorescent analogue of propranolol (*N*-[-2-hydroxy-3-naphthoxy propyl]-*N′*-[-9-amino acridin] isopropyl diamine) (Fig. 1). Its spectroscopic molar extinction coefficient, E_{260}, in water is 1.07×10^5. The inhibitory effect of 9-AAP was calculated from the concentration of this compound which was required to inhibit 50% of the (−)-adrenaline stimulated activity in a β-receptor-dependent adenylate cyclase system. Consequently, the dissociation constant of 9-AAP was found to be $(3\pm1)\times10^{-8}$ M (Fig. 2).

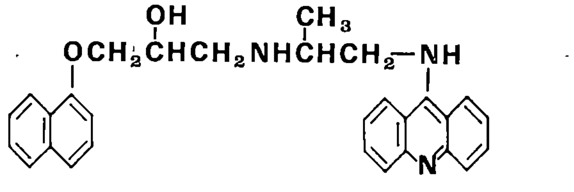


Fig. 1 The structure of 9-amino-acridin-propranolol.

9-AAP in saline (2.5 mg kg^{-1}) was administered by slow injection into the tail veins of albino rats (200–220 g). Control animals were pretreated with one of the following compounds: (±)-propranolol, (−)-propranolol, or (+)-propranolol (5 mg kg^{-1} in saline) by slow intravenous injection. Thirty minutes later 9-AAP (2.5 mg kg^{-1}) was administered to each of the control animals. All the animals were killed by decapitation under light ether anaesthesia 30 min after injection of 9-AAP. The brains of all animals were quickly removed. The cerebellum of each animal was separated, immersed in "tissue OCT compound" and frozen in liquid nitrogen. Later, 6–8-μm coronal,

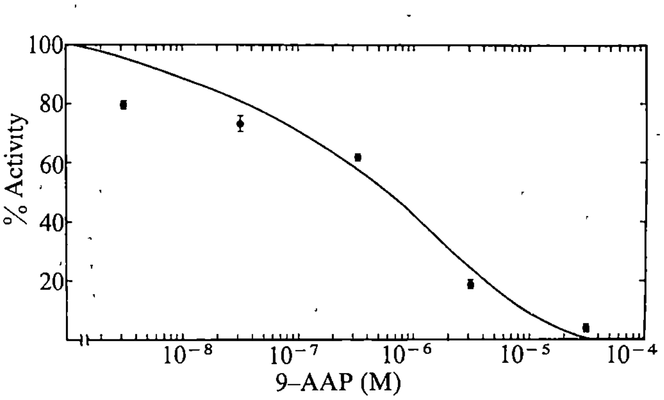


Fig. 2 Inhibition of (−)-adrenaline-stimulated activity of adenylate cyclase by 9-AAP. The dissociation constant (K_D) was calculated as follows[16,17]:

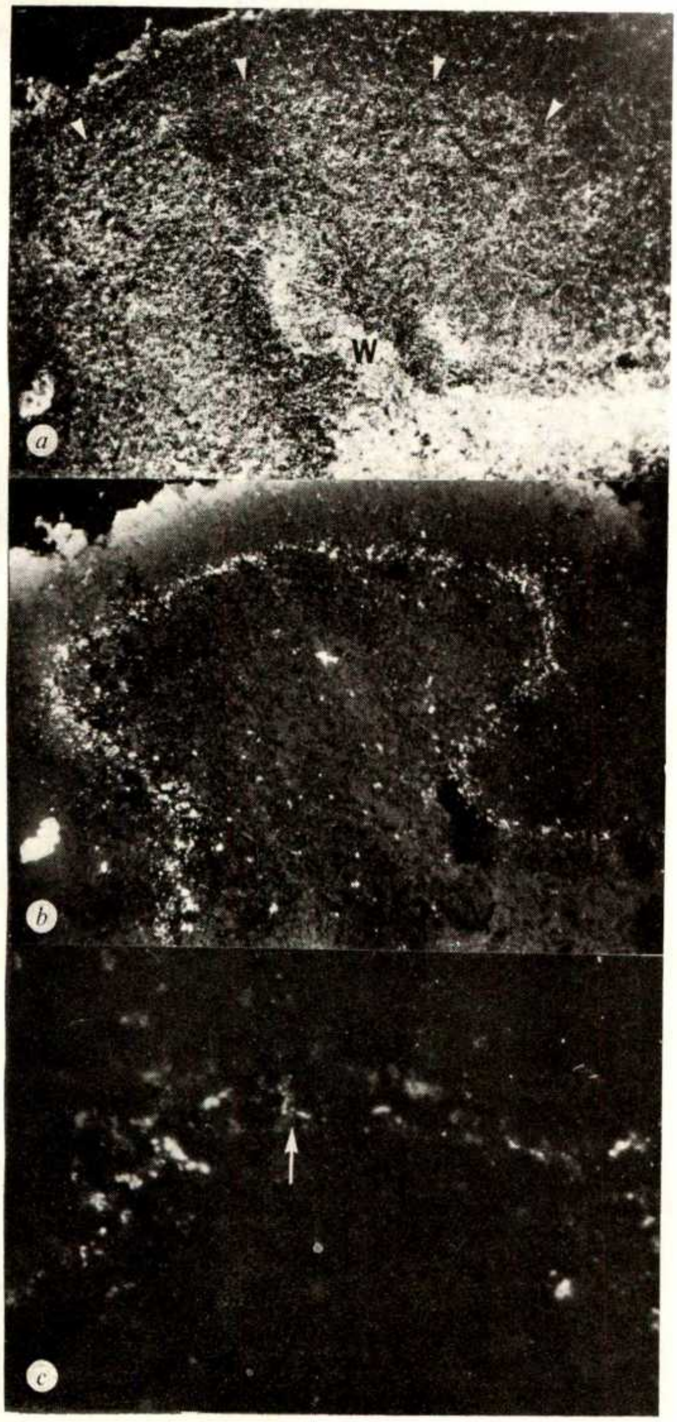
$$K_D = \frac{S_{50}}{L}\times K_L$$

where S_{50} is the concentration of 9-AAP which causes 50% inhibition of total (−)-adrenaline activity; L is the concentration of (−)-adrenaline in the cyclase assay; K_L is the dissociation constant of (−)-adrenaline to the β receptor (10^{-5} M). $S_{50} = 6\times10^{-7}$ M; $K_D = 3\times10^{-8}$ M.

sagittal and transverse cerebellar sections were cut in a cryostat at −20 °C. The frozen sections were mounted on glass slides and air dried. Sodium phosphate buffer, 0.08 M, pH 7.4 was applied and coverslips were placed in position. The sections were visualised under phase contrast and transmitted ultraviolet illumination on a Zeiss Universal Fluorescent Microscope with HBO 200-W super pressure mercury lamp, exciter filter UG1 and barrier filter No. 41.

Dense concentration of 9-AAP binding sites was observed in every section as a well defined yellow fluorescent belt in the cerebellar cortex. This phenomenon was localised along a line between the molecular and granular cell layers which corresponds anatomically to the Purkinje cell layer (Fig. 3*a* and *b*). Purkinje cell bodies and their major apical dendrites could be delineated by phase-contrast illumination. The highest intensity of fluorescence was observed on the apical dendrites of Purkinje cells. In most instances the distribution pattern of 9-AAP on these dendrites appeared as threads of bead-like fluorescent sites, placed at quite regular intervals from each other (Fig. 3*c*).

Fig. 3 *a*, Phase-contrast photomicrograph of sagittal section of rat cerebellum. W, white matter. The location of the Purkinje cell layer is indicated by arrows (frozen section, ×30). *b*, Fluorescent photomicrograph of the same region. Intense fluorescence is observed within the Purkinje cell layer (frozen section, ×30). *c*, High magnification fluorescent photomicrograph of Purkinje cell layer. Bead-like fluorescent sites are seen along apical dendrites of Purkinje cells and their bifurcations (frozen section, ×100).



Less often, coalescence of these sites into larger aggregates was observed. Sparse distribution of faint fluorescent threads and dots was observed within the inner portion of the molecular cell layer. The latter finding could represent 9-AAP binding sites on fine ramifications of Purkinje cell dendrites within the molecular layer. Occasionally fluorescent spots were observed within the granular cell layer and the cerebellar white matter. Fluorescence was practically absent in cerebellar sections from control animals pretreated with (±)-propranolol and (−)-propranolol. In sections from control animals pretreated with (+)-propranolol, the distribution pattern of fluorescence was similar to that observed in animals which were treated by 9-AAP alone, though reduced in intensity.

It seems unlikely that the discrete anatomical localisation of the fluorescent compound in the Purkinje cell layer of rat cerebellum was due to nonspecific tissue binding. *In vitro* studies show that 9-AAP exhibits an affinity for β-adrenoceptor sites (Fig. 2). In addition, stereospecific blocking of 9-AAP binding sites was demonstrated by pretreatment with (±)- and (−)-propranolol but not with (+)-propranolol. The decreased intensity of fluorescence which was observed in cerebellar sections of animals pretreated with (+)-propranolol was probably due to low specific activity of the (+) isomer or to its contamination by small amounts of (−)-propranolol[12]. Furthermore, the distribution pattern of 9-AAP binding sites correlates with the previously described localisation of the presynaptic NA-containing nerve terminals in the rat cerebellar cortex. These fibres were found in the Purkinje and molecular cell layers where they synapse with the major apical dendrites and with further dendritic processes of Purkinje cells, respectively[6,7]. These data, in conjunction with the previous indirect evidence for the presence of β-adrenoceptors in Purkinje cells[8–10,13–15], suggest that the distribution of 9-AAP binding sites may correspond to β-adrenoceptor sites in rat cerebellum.

Direct binding with 9-AAP may provide a useful *in vivo* fluorescent method for the study of the postsynaptic component of NA synapses. Further studies which utilise this compound for the detection of β-adrenoceptor in other regions of the CNS and various non-neural tissues are currently in progress.

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Spontaneous electrical potentials and pituitary hormone (MSH) secretion

CELLULAR secretions from either exocrine or endocrine glands are regulated in most cases by hormonal or neurohormonal agents. These chemical messengers interact with cellular receptors to stimulate a particular secretory response. In general, it would seem that changes in the transmembrane electrical potential of the secretory unit is in some way intimately involved in this stimulus–secretion coupling[1]. Here we provide evidence of cellular secretion resulting from spontaneous electrical activity (most probably depolarisations) after removal of a previously inhibitory stimulus.

Melanophore stimulating hormone is synthesised and released from the pars intermedia of the vertebrate pituitary[2]. This hormone has an important role in integumental colour changes of most vertebrate species[3]. MSH secretion from the vertebrate pituitary is regulated, as for other pituitary hormones, by hypothalamic control[2]. Transplantation of the pituitary gland to an ectopic site or to an *in vitro* condition results in a continuous uninhibited release of MSH[4] and prolactin[5]. The *in vitro* secretion of these hormones is reversibly inhibited by a variety of experimental methods demonstrating that such manipulations are not themselves responsible for this enhanced hormone secretion[5,6]. Thus, MSH and prolactin secretion are unique in that isolation from a hypothalamic inhibitory influence results in active, spontaneous secretion of these hormones. Other cell types require exogenous stimulatory cues to enhance secretion.

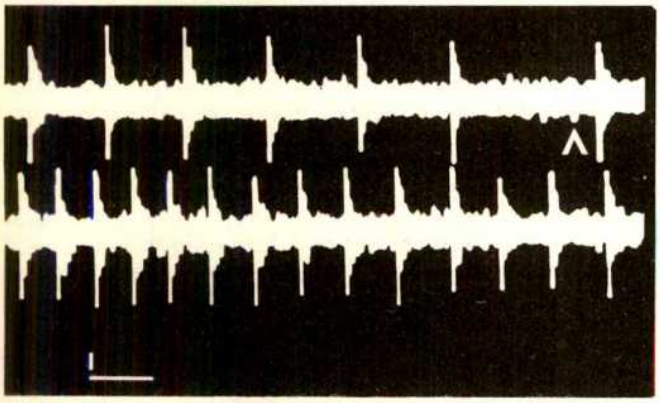

Fig. 1 Continuous oscilloscope recording of isolated neurointermediate lobe from *R. pipiens* pituitary. The top sweep indicates extracellular spontaneous electrical activity from a single unit in the pars intermedia. At the time indicated by the arrow, a 0.8 V, 10 ms pulse from an electronic stimulator was passed through the gland by way of two silver–silver chloride electrodes flanking the pars intermedia. The bottom trace shows the increase in frequency of spikes occurring approximately two seconds later with twice as much activity as the spontaneous sweep and lasting for about 30 s. This enhancement of electrical activity is correlated with an increase in hormone secretion (Fig. 3). The vertical amplitude scale is 25 mV and the horizontal time scale is 150 ms.

Although several models for the neuronal control of pars intermedia cell function have been provided[2,7], the physiological basis for the autonomous secretion of MSH in the absence of hypothalamic control is unresolved. As was earlier suggested for the prolactin cell of the pars distalis[5], MSH secretion may result from a spontaneous depolarisation of pars intermedia cells. Evidence that such a hypothesis may be correct is presented here.

Neurointermediate lobes of the frog, *Rana berlandieri forreri* (*Rana pipiens, sensu lato*) were removed and incubated in tissue culture for one to two weeks. The neurointermediate lobes were cultured to be reasonably certain that when recording electrical activity of the pars intermedia cells, injury potentials set up by cut axonal endings would not be present and be confused with the electrical activity of the pars intermedia cells. Interneurones are apparently absent from the pars intermedia[8]. To further insure against such a possibility, the intermediate lobes in some experiments were incubated in the presence of tetrodotoxin (TTX) which is known to inhibit action potentials in nerves. Tetrodotoxin (10^{-6} M) did not block electrical activity or inhibit MSH secretion.

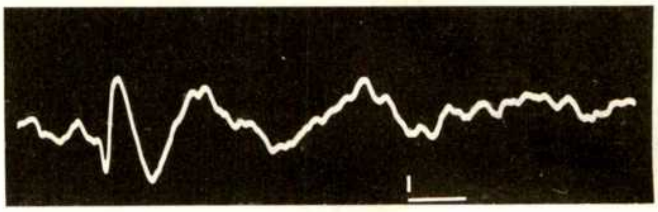

Fig. 2 Fast oscilloscope sweep showing the shape of a single spike unit as in Fig. 1. The triphasic (positive–negative–positive) wave is characteristic of a depolarisation recorded extracellularly from the pars intermedia. Triphasic pulses are generally associated with conduction of excitation along nerve axons. Similar pulses recorded in these experiments are difficult to explain except perhaps that potentials travelling through cells in the pars intermedia may be electrically coupled. As a control, no recordings could be found in the pars nervosa cultured *in vitro*. The vertical amplitude scale is 25 mV and the horizontal time scale is 10 ms.

Spontaneous electrical activity (Fig. 1, top) was recorded with 3 M KCl-filled glass micropipette electrodes (0.5–1.5 μm diameter, 10–30 MΩ resistance) from just inside the periphery of the gland down to about 300 μm. A silver reference electrode was placed in the bathing amphibian Ringer's solution. Although quite often the tip of the electrode would penetrate a cell with a resulting rapid negative deflection, no steady intracellular potentials could be recorded for any length of time. Instead, extracellular spikes (as shown in Fig. 2) indicate characteristically depolarising potentials with triphasic events (positive–negative–positive, with major phase mostly negative). Whenever a fairly steady frequency of spikes was found, a 0.8 V, 10 ms single pulse of electricity was sent through the gland by way of two silver–silver chloride stimulating electrodes flanking opposite sides of the neurointermediate lobe. In about 75% of the more than 90 cases studied, the electrical stimulation tended to increase the frequency of spikes two- to threefold for as long as 60 s (Fig. 1, bottom) while the remaining 25% maintained the same frequency or showed inhibited frequency. The latency between stimulation and onset of frequency changes varied between a half to a few seconds and very possibly the range might extend further in either direction. The latency between a single electrical stimulus and the increase in frequency of spontaneous pulses as well as the duration of the after-discharges are much greater than would be expected if the pulses were of neuronal origin. Thus, in the majority of cases, spontaneous

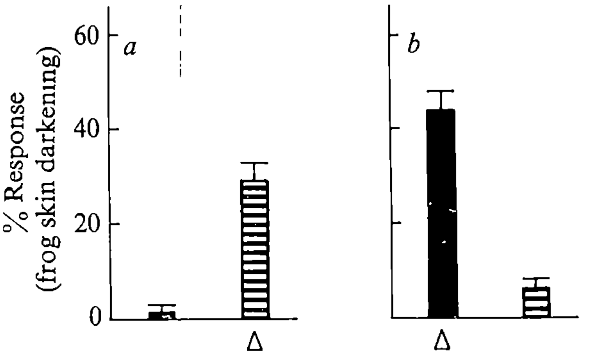

Fig. 3 Bioassay of MSH secreted from isolated, tissue-cultured frog neurointermediate lobes. Eight glands were used and were divided into two experimental groups After an initial 30-min preincubation (to equalise secretion rates in both groups), one group of glands (arrow, *a*) was subjected to 0.8 V, 10 ms pulses at 1 pulse s^{-1} for 60 min. The other group of glands remained as a control without stimulation. Then, in a subsequent incubation, a similar stimulation was applied to the previously untouched group of glands (arrow, *b*) and the other previously stimulated glands served as controls. Note the correlation between electrical stimulation and enhancement of MSH secretion and reversal of secretion when stimulation is terminated. Each value represents the mean ± s.e., darkening response of the frog skins (8 per group) used to bioassay the released MSH[16].

electrical activitity of pars intermedia cells is enhanced through electrical stimulation.

Figure 3 shows the relative quantities of MSH released during electrical stimulation of tissue-cultured neurointermediate lobes. These results indicate that electrical stimulation reversibly enhances MSH secretion. The electrophysiological readings and the bioassay of released MSH were repeated several times with the same conclusion using both tissue-cultured glands and freshly excised glands. Similar data (electrically enhanced pulse frequency and enhanced MSH secretion) in our preliminary experiments using the isolated rat pituitary gland have been obtained.

These results suggest that MSH secretion from the isolated frog or rat pituitary may result from spontaneous depolarisations of pars intermedia cells. Since Ca^{2+} is required for MSH secretion[2], depolarisation of the cell membrane may act to increase the permeability to this ion and activate the secretion process. Evidence that depolarisation itself leads to enhanced MSH secretion is shown by the demonstration that electrical stimulation of isolated neurointermediate lobes results in reversible enhancement of hormone secretion. The results demonstrate a unique mechanism of pars intermedia cell secretion possibly only shared by a similar mechanism for prolactin release in the pars distalis. Whereas enhanced cellular secretion above basal levels from other cellular species is induced by changes in membrane potential resulting from hormonal, neurohormonal or pharmacological stimulation, secretion of MSH results spontaneously when the pars intermedia cells escape from hypothalamic regulatory cues and depolarise.

The nature of the hypothalamic control of MSH secretion is at present debatable. Both peptide factors of hypothalamic origin and direct innervation of the pars intermedia by hypothalamic neurones are considered to regulate MSH secretion. There is clear morphological evidence for a control of pars intermedia cell function by direct innervation[9]. Light[9] and electron microscopic evidence[10,11], favours the view that the inhibitory effect of these neurones is mediated by a catecholamine. The physiological support for such morphological evidence was provided by the demonstration that catecholamines such as noradrenaline, adrenaline and dopamine inhibit MSH secretion reversibly *in vitro*[12,13]. The receptor basis for the mechanism of action of these catecholamines has also been provided[13].

Our results provide evidence that inhibition of MSH secretion is maintained by a tonic polarisation of the individual pars intermedia cells. Photostimulatory cues of ocular and possibly epithalamic (possibly pineal) origin modulate or otherwise influence the hypothalamic input to the pars intermedia[8,14]. It has been suggested that each pars intermedia cell may be innervated by an inhibitory neurone. Inhibition of these neurones might be accomplished by other neurones, possibly directly or by presynaptic relationships. Variable activity of the latter neurone would modulate the activity of the tonically inhibitory neurone[11,14]. In the absence of the inhibitory polarising influence on the pars intermedia cells, they would spontaneously depolarise and this would result in the release of MSH. Although a number of secretory systems have been studied[1] (pars distalis of pituitary, salivary glands, exocrine pancreas), we know of no other example where cellular secretions may be initiated by such spontaneous depolarisations. The pars intermedia cell, therefore, offers a unique model system for studying various electrophysiological parameters.

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Evidence for analgesic activity of enkephalin in the mouse

HUGHES *et al.*[1] have recently described the identification and structure elucidation of methionine- and leucine-enkephalin, the postulated endogenous morphine-like factors from mammalian brain[2–4]. The structures were confirmed by comparing the mass spectra and electrophoretic mobilities of natural and synthetic material and their biological activities established *in vitro* in preparations of the mouse vas deferens and the guinea pig ileum. We have synthesised the two pentapeptides H–Tyr–Gly–Gly–Phe–Met–OH and H–Tyr–Gly–Gly–Phe–Leu–OH, as well as two analogues (desamino–Tyr)–Gly–Gly–Phe–Met–OH and H–Tyr–Gly–Gly–OH and compared their biological activities. Our results indicate that the two natural compounds induce analgesia in mice, whereas the two analogues are devoid of such activity.

The pentapeptides were built up by fragment condensation methods[5] (mixed anhydride or *N*,*N*′-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole). Boc–Tyr–OH was coupled with H–Gly–Gly–OBz to give Boc–Tyr–Gly–Gly–OBz (melting point 97–102 °C, $[\alpha]_D^{20} = -5.4°$, $c = 2$ in DMF). After catalytic hydrogenation, Boc–Tyr–Gly–Gly–OH was reacted either with H–Phe–Met–OMe or H–Phe–Leu–OEt to produce Boc–Tyr–Gly–Gly–Phe–Met–OMe (melting point 92 °C, $[\alpha]_D^{20}$

= −20.2°, $c = 2$ in DMF) and Boc–Tyr–Gly–Gly–Phe–Leu–OEt (melting point 95 °C, $[\alpha]_D^{20} = -16.1°$, $c = 2$ in DMF). After saponification of the esters, the Boc– groups were cleaved by trifluoroacetic acid. The two natural enkephalins were obtained as homogenous crystalline compounds after transforming the peptides to a salt free form on an ion exchange column (methionine-enkephalin, melting point 190–196 °C, $[\alpha]_D^{20} = +26.1°$, $c = 1$, 95% AcOH; leucine-enkephalin, melting point 157–167 °C, $[\alpha]_D^{20} = +26.4°$, $c = 1$, 95% AcOH). The desamino-tyrosine analogue of leucine-enkephalin was synthesised in a similar manner starting from 3-(4-hydroxyphenyl) propionic acid. All peptides gave correct amino acid analysis.

The analgesic effects of methionine- and leucine-enkephalin were studied using the tail flick test in mice[6]. A direct comparison with morphine using the same pretreatment interval was complicated by their different pharmacokinetic properties. Morphine (0.56–3.2 µg per mouse) had a slow onset of action following intracerebroventricular administration, peak activity being reached after 15 min. In contrast the two endogenous enkephalins were maximally active after 2 min and had a duration of action of barely 5 min. This difference was not unexpected since enkephalin is readily destroyed enzymatically[2,4]. Doses of morphine that had an analgesic effect within 2 min were toxic at later observation times.

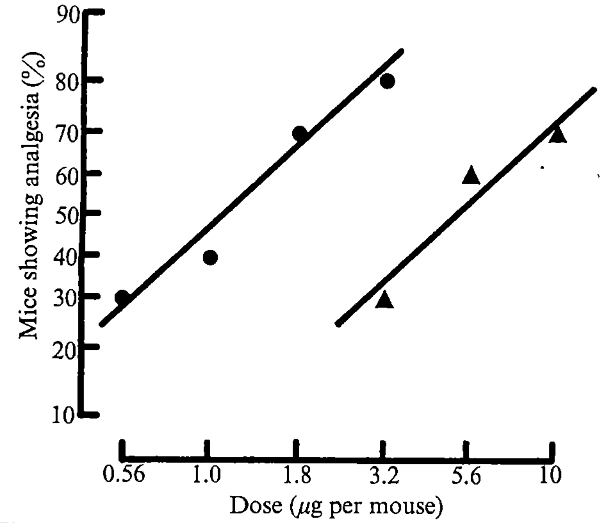

Fig. 1 The analgesic effect of morphine in the tail flick test[6] in the mouse 15 min after intracerebroventricular administration alone (●) and following pretreatment with 0.1 mg kg^{-1} subcutaneous naloxone (▲). 10 animals per dose.

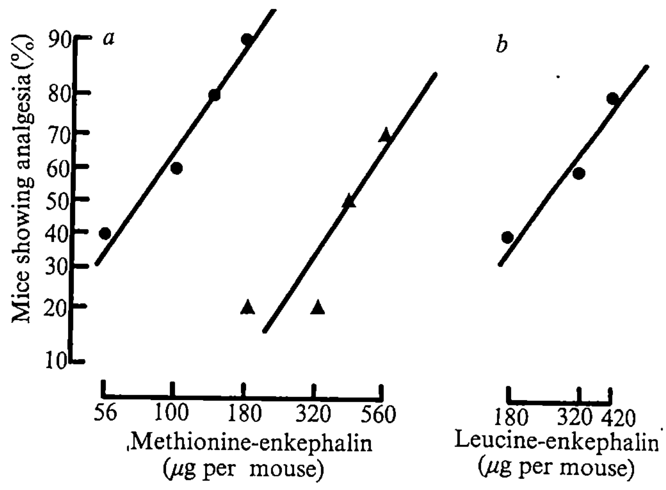

Fig. 2 The analgesic effects of methionine- and leucine-enkephalin in the tail flick test[6] 2 min after intracerebroventricular administration alone (●) and following pretreatment with 0.1 mg kg^{-1} subcutaneous naloxone (▲) 10 animals per dose.

The dose–response curves of the analgesic effect of morphine at 15 min and of the two enkephalins at 2 min were approximately parallel (Figs 1 and 2). Morphine was 75 and 240 times more active than methionine- and leucine-enkephalin respectively (Table 1). Naloxone (0.1 mg kg^{-1} subcutaneously), administered 20 min before the measurement of analgesia, inhibited the analgesic effects of methionine-enkephalin and morphine in a competitive manner, the curves being displaced to the right by a factor of about 5 (Figs 1 and 2*a*). This suggested that the two compounds were affecting the same system in a similar manner. In contrast, the analgesic effect of leucine-enkephalin was not inhibited by pretreatment with naloxone except at the very high dose of 10 mg kg^{-1} subcutaneously. The different susceptibilities of the two enkephalins to blockade by naloxone could indicate that the leucine analogue may behave as a mixed agonist–antagonist at the opiate receptor[9], whereas the methionine form may more closely resemble morphine in its action. The results may explain why Hughes *et al.*[10] required significantly more naloxone to antagonise the effects of the unseparated enkephalin than to inhibit the effect of normorphine *in vitro*.

The maximum effect of morphine (1.0–3.2 mg kg^{-1}), administered intravenously, occurred after 15 min (ED_{50} =2.0 mg kg^{-1}). Methionine-enkephalin exhibited a transient effect at very high doses (ED_{50} = 170 mg kg^{-1} intravenously), analgesic activity being detected only within the first 15 s of administration. Leucine-enkephalin was only weakly active (4 out of 10 mice showing analgesia) at the highest soluble dose (320 mg kg^{-1} intravenously). These results obtained *in vivo* with the two enkephalins following intracerebroventricular and intravenous administration support the *in vitro* finding[1] that the methionine analogue is more active than the leucine form.

In binding studies using rat brain homogenate[11], methionine-enkephalin at 25 °C was twelve times less potent than morphine in inhibiting specific ^{3}H-naloxone binding in a sodium-free medium and approximately six times less potent in a medium containing sodium (Table 2). Hughes *et al.*[1] claimed a higher affinity for enkephalin than morphine but they used guinea pig and not rat brain homogenate. Furthermore, the temperature at which incubation and filtration is carried out has a pronounced effect on the binding of opiates[12] which might be responsible for the discrepancy between the results.

Table 1 Effect of naloxone pretreatment on analgesic activity of morphine and enkephalin

Test substance	Time (min) after administration	ED_{50} of analgesic activity µg per mouse intracerebroventricularly ($P = 0.05$) Alone	After naloxone
Morphine–HCl	15	1.1 (0.7–1.8)	5.2 (3.2–8.3)
H–Tyr–Gly–Gly–Phe–Met–OH	2	75 (52–100)	420 (305–579)
H–Tyr–Gly–Gly–Phe–Leu–OH	2	240 (159–363)	180 (115–282)
(des–NH_2–Tyr)–Gly–Gly–Phe–Leu–OH	2	> 32 (toxic)	—
H–Tyr–Gly–Gly–OH	2	$\gg$ 320	—

ED_{50} values ($P = 0.05$) for the analgesic activity in the tail flick test in the mouse alone and after pretreatment with naloxone (0.1 mg kg^{-1} subcutaneously). The ED_{50} is defined as the dose that prolonged the reaction time in seconds by more than 75% in half of the mice. 10 animals were used per dose and drugs administered intracerebroventricularly (10–30 µl per mouse) were dissolved in artificial cerebrospinal fluid[7]. The accuracy of the intracerebroventricular injections, performed using the method of Haley and McCormick[8], were confirmed macroscopically with the aid of Indian ink

Table 2 Binding of ^{3}H-naloxone by rat brain

Test substance	IC$_{50}$ of specific ^{3}H-naloxone binding No NaCl nM	 100 mM NaCl nM	IC$_{50}$ ratio +NaCl/−NaCl
Morphine–HCl	4.5±0.6 (3)	73±10 (3)	16.2
Pentazocine	12.3±0.3 (2)	87± 3 (2)	7.1
H–Tyr–Gly–Gly–Phe–Met–OH	55.4±9.4 (5)	412±69 (5)	7 4
H–Tyr–Gly–Gly–Phe–Leu–OH	224 ±19 (5)	1,075±75 (5)	4.8
(des NH$_2$–Tyr)–Gly–Gly–Phe–Leu–OH	> 10,000	> 10,000	—
H–Tyr–Gly–Gly–OH	> 10,000	> 10,000	—

IC$_{50}$ values for the inhibition of specific ^{3}H-naloxone binding in rat brain homogenate. The IC$_{50}$ is defined as the concentration of substance required to inhibit by 50% specific binding of ^{3}H-naloxone (nM) to preincubated (30 min at 37 °C) homogenates of rat brain minus cerebellum in 0.05 M Tris buffer (*p*H 7.4 at 25° C)[12] Incubations in triplicate were assayed at 25 °C for 40 min alone or in the presence of 100 mM NaCl. Filtrations were performed at 25 °C. The values are the mean ± s.e.m.; the number of experiments is given in parentheses. The "sodium response ratio" is defined as the ratio of the IC$_{50}$ values in the presence and absence of sodium[11].

The low "sodium response ratio" calculated for leucine-enkephalin is characteristic for a dual agonist–antagonist at opiate receptors[11] and corresponds with the findings *in vivo*. On the other hand, the similarity between the sodium response ratio of methionine-enkephalin and the partial agonist, pentazocine, was surprising and suggests that methionine-enkephalin might also possess antagonistic activity. Further studies are in progress to examine in greater detail the opiate receptor blocking activities of the two enkephalins and their stabilities during incubation.

The desamino analogue in non-toxic doses and the tripeptide did not produce analgesia following intracerebroventricular administration (Table 1) and had no affinity for the opiate receptor in the binding studies (Table 2). The results with the former compound show the terminal amino group of enkephalin to be indispensable for its morphine-like activity and suggest that it corresponds to the nitrogen atom of morphine. The inactivity of the tripeptide shows that the minimal requirements for analgesic activity are not met by the first three amino acids of enkephalin.

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Effects of microiontophoretically applied methionine-enkephalin on single neurones in rat brainstem

THE identification of a specific opiate receptor in mammalian brain[1], and also of an endogenous morphine-like factor, enkephalin, which possesses potent opiate agonist activity peripherally[2], raises the question of the possible physiological significance of this substance in the central nervous system. There is evidence from a number of studies suggesting that the brainstem is an important site for antinociceptive action[3–5]. In previous studies[6], morphine applied iontophoretically to single neurones in the rat brainstem, has been found to cause either an increase or a decrease in firing rate, but only the depressant response showed stereospecificity and could be blocked by the specific opiate antagonist, naloxone[7,8]. Similar findings on cortical neurones have been reported[9]. We have therefore investigated the effects of iontophoretically applied methionine-enkephalin (met-enkephalin), the more potent peripherally of the two pentapeptides, on the activity of single neurones in the rat brain stem and compared these effects with those of morphine, and also of another narcotic analgesic, etorphine, which is considerably more potent. Since naloxone has been shown to block peripheral effects of enkephalin[10], we have examined its effects on the neuronal responses to met-enkephalin, morphine and etorphine. Our findings support the concept of a physiological role for endogenous enkephalin in the central nervous system.

Table 1 Comparison of effects of enkephalin with either morphine or etorphine applied to same neurone

	Morphine				Etorphine	
Effect	+	NE	−	+−	NE	−
+	9	0	0	0	0	0
Enkephalin (NE)	0	7	0	0	2	0
−	14	10	10	3	0	8
−+	1	0	1	0	0	0

Numbers indicate number of neurones in each case.

+, Excitation; −, inhibition; NE, no effect; +− excitation followed by inhibition; −+, inhibition followed by excitation.

Drugs were applied from five-barrelled glass micropipettes inserted into the medullary reticular formation of rats under light urethane anaesthesia (see ref. 6 for further experimental details). Met-enkephalin (5 mM, *p*H 5.0) supplied by Dr B. A. Morgan of Reckitt & Colman, and etorphine HCl (24 mM, *p*H 5.0) were dissolved in distilled water. Other drug solutions were as described previously[7].

The effects of met-enkephalin, applied iontophoretically, for periods of 5–90 s with positive currents of 10–45 nA were studied on 82 spontaneously active neurones in 21 rats. Enkephalin depressed the activity of 70% (58) of the neurones tested (Fig. 1*a*) and excited 11% (9). Biphasic effects were occasionally seen (4) but whereas the biphasic response to morphine consisted of excitation followed by inhibition, that to enkephalin was consistently inhibition followed by excitation. In Table 1 the effects of enkephalin are compared with those of either morphine or etorphine, applied to the same neurone. When morphine or etorphine produced depression, so did enkephalin. On the other hand, although enkephalin mimicked some of the excitatory responses to morphine, in many cases it depressed neurones which were excited by morphine. There were also some

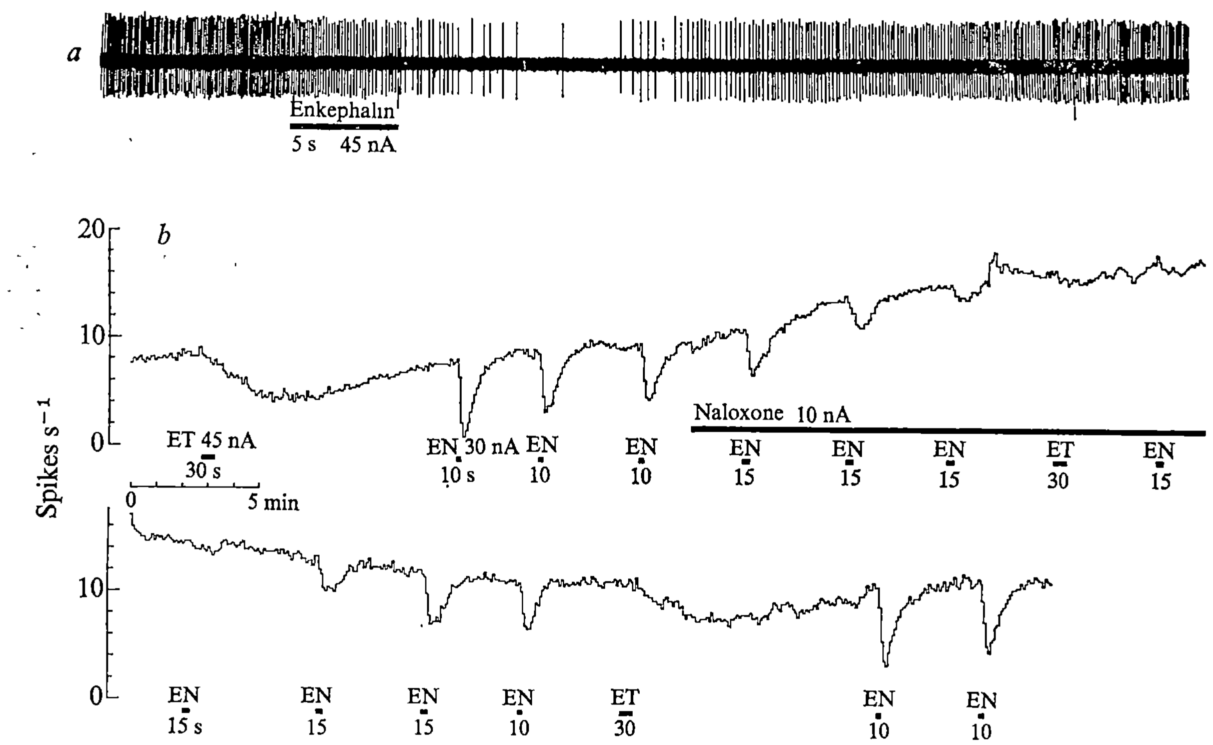

Fig. 1*a*, Inhibitory effect of iontophoretically applied enkephalin (45 nA for 5 s; current balancing was used) on the spontaneous activity of a rat medullary neurone. The inhibitions caused by enkephalin were usually slow in onset, reaching a maximum effect 10–40 s after the start of application. and recovering slowly over 1–3 min from the end of application *b*, Antagonism by naloxone of the inhibitory actions of etorphine (ET) and enkephalin (EN) on the firing rate (spikes s^{-1} over consecutive 5-s epochs) of a rat medullary neurone. Periods of iontophoretic applications are indicated by the bars; expelling currents (balanced; nA) are shown above, and durations of applications below the bars. Note that during and after naloxone application, longer enkephalin applications were used, but in spite of this, the effects of enkephalin and of etorphine were blocked; gradual and complete recovery was observed.

neurones, depressed by enkephalin, which seemed to be unaffected by application of morphine. Unlike morphine, etorphine was never observed to increase neuronal activity, and therefore the correlation between the effects of enkephalin and those of etorphine was much better.

Naloxone was applied iontophoretically, using currents of 5–30 nA and blocked enkephalin-induced depression 8 out of 8 times (Fig. 1*b*). In two of these, morphine depression was also blocked by naloxone and in another two etorphine depression was blocked. In a single neurone which was excited by both morphine and enkephalin, naloxone failed to block either response. Naloxone sometimes had effects on firing rate but these were unrelated to its antagonistic actions.

It would seem that in the brainstem the depressant action of enkephalin is likely to be more important than its excitatory action since this response was reversed by naloxone. This is in good agreement with the previous finding that only the depressant action of morphine was antagonised by naloxone. Note that etorphine, a more potent analgesic drug, produced only depression of neuronal activity and this too was antagonised by naloxone. Whether or not the depressant response to enkephalin can be taken to indicate a physiological action of this substance on these neurones, remains to be seen. Until more information is available on the distribution of enkephalin at the subcellular level, this will be an open question.

L.A.L. is an MRC scholar.

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Effects of methionine-enkephalin and leucine-enkephalin compared with those of morphine on brainstem neurones in cat

Two new peptides, methionine-enkephalin (met-enkephalin) and leucine-enkephalin (leu-enkephalin), have been isolated from brain, identified and synthesised[1]. The interest in these compounds lies in their morphine-like activity which was recognised and tested by their action on isolated preparations from the peripheral nervous system. Since morphine is used for its central action in producing analgesia, it is important to investigate whether these substances have activity in the central nervous system similar to that of morphine. We report here the findings of similar depressant actions of all three substances on brainstem neurones in the cat.

To compare the actions of the enkephalins and morphine we applied them to single neurones in the brainstem of unanaesthetised, decerebrate cats by iontophoresis from five- or seven-barrelled micropipettes. Pipettes were inserted through the floor of the IVth ventricle 2–7 mm rostral to the obex and 0–1.5 mm lateral from midline. Histological sections showed that the pipettes sampled

Table 1 Percentages of brainstem neurones showing inhibition, a biphasic effect of inhibition followed by excitation, or no effect, in response to the iontophoretic application of enkephalin or morphine

	Inhibition	Biphasic	No effect
Met-enkephalin ($n = 96$)	89	1	10
Leu-enkephalin ($n = 40$)	92.5	0	7.5
Morphine ($n = 84$)	87	2	11

mainly the activity of neurones in nucleus reticularis gigantocellularis and pontis caudalis. The substances, which were usually applied with currents of 10–30 nA for 30-s periods, were tested for effects on spontaneous activity or, when this was low or absent, on activity induced by the continuous application of homocysteic acid (0–17 nA).

The predominant effect of met- and leu-enkephalin and morphine was depression of neuronal firing rate as shown in Fig. 1. Rarely, the depression was followed by excitation, but we have not observed any instances of excitation alone. A few units did not show any response, and the incidence of the different effects is given in Table 1. In 81 out of 84 cells in which the effects of met-enkephalin and morphine were compared, their effects were qualitatively the same (73 inhibited, in one of which the inhibition was followed by excitation, and 8 unaffected). The effects of met- and leu-enkephalin began within the first 5 s of the ejection period and usually reached a peak by 15–20 s.

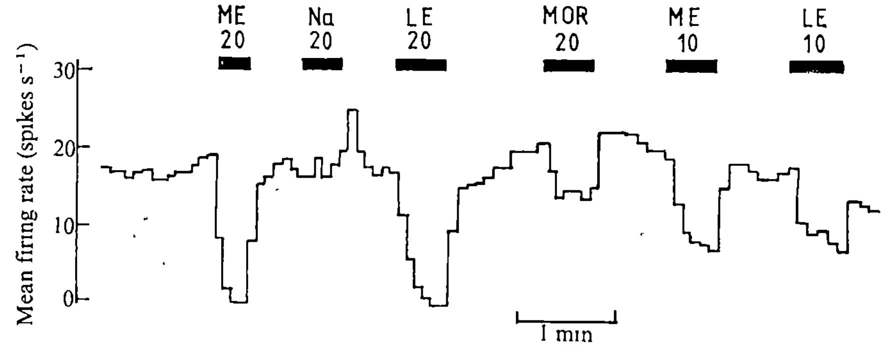

Fig. 1 Effects of iontophoretically applied met-enkephalin (ME), leu-enkephalin (LE) and morphine (MOR) on the firing rate, induced by a continuous current of 9 nA of DL-homocysteic acid, of a brainstem neurone. The application of Na^+ served as a control for the effects of current. Applications are indicated by the horizontal bars. Currents are shown in nA. Firing rate shown in successive 5-s epochs. Met- and leu-enkephalin were supplied by Dr B. A. Morgan of Reckitt & Colman; they were dissolved at *p*H 4.5, at 0.016 M. Morphine was used as the sulphate, at *p*H 4.5, 0.025 M. One barrel of the pipette contained DL-homocysteic acid at *p*H 8.5, 0.1 M, and another contained 2 M NaCl to control for current effects. In some experiments a solution of naloxone (Endo Laboratories), 0.027 M at *p*H 4.5, was used in one barrel.

When cells which were not firing spontaneously were fired by the application of homocysteic acid, enkephalin and morphine had pronounced depressant actions, suggesting that their effects were probably exerted on the cell membrane rather than presynaptically.

The effects of met- and leu-enkephalin could often be seen with low ejection currents (5 or 10 nA), and the degree of inhibition was similar for both compounds. Thus leu-enkephalin, compared with met-enkephalin, seems to be more effective in the brain than it was found to be at peripheral receptors[1]. In most cases higher currents were required to obtain the same effects with morphine. A reliable comparison of potencies, however, cannot be made since the relative efflux rates of these substances are not known and only a method such as that described by Gent *et al.*[2] would enable them to be determined.

Effects on reticular neurones are of interest since they respond to noxious stimulation[3,4]. These cells show a wide convergence of afferent input[5], and in some of the present experiments reticular neurones were identified by their response to pressure or pinch over large areas of the body surface[4]. Met-enkephalin was tested on 22 of these neurones —(21 inhibited, 1 unaffected). Morphine inhibited all 21 and leu-enkephalin all 13 on which they were tested. Thus reticular neurones with somatic responses in the medulla or caudal pons were consistently inhibited by all three substances.

The effects of morphine on a variety of systems can be antagonised by naloxone. This drug also blocks the inhibition of brain stem neurones by morphine[6], and it has now been shown also to block the inhibitory actions of met-enkephalin on brainstem neurones in the rat[7]. In the cat naloxone has been reported to antagonise the analgesia produced by bulbar raphe stimulation[8]. It was therefore surprising to find that neither the effects of morphine nor those of met-enkephalin on brainstem neurones in the cat could be blocked by naloxone. Although naloxone was applied for periods up to 35 min with currents of up to 150 nA (means 10 min and 81 nA), no appreciable antagonism was observed in the 16 neurones on which it was tested.

This result might suggest that the action of morphine on the neurones (including reticular neurones) which were investigated in the present experiments is unrelated to its analgesic action. Unfortunately naloxone does not, however, seem to have been tested for effects on analgesia produced by morphine in the cat. (There is a widely held belief that morphine is not analgesic in the cat, but doses of 1–2 mg kg^{-1} intravenously have been reported to produce analgesia[9]; this action occurs at a much lower dose when the injections are made into the IVth ventricle[10], suggesting that it is produced by an action on the brainstem.)

To conclude, both met- and leu-enkephalin have inhibitory actions similar to those of morphine on neurones in the brainstem of the cat, but surprisingly none of these effects can be blocked by naloxone. Thus the receptors responsible for these inhibitory actions must be different from those in the rat brainstem, and those in peripheral systems which can be antagonised by naloxone.

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Reduction of possible hazards in the preparation of recombinant plasmid DNA

THE preparation of plasmid DNA requires the collection of plasmid-containing bacteria, usually by centrifugation of liquid cultures. This step is a potential source of laboratory contamination, especially when large volumes, requiring continuous flow centrifugation, are processed.

We report here that bacteria killed by treatment with chloroform[1] or with diethylpyrocarbonate[2] are as good a starting material for the purification of plasmid DNA as are untreated bacteria. For these experiments we have used a streptomycin-dependent strain of *Escherichia coli* as recipient in transfection experiments and as host for the amplification of plasmid DNA.

We tested several strains of streptomycin-dependent *E. coli* (N543, N530 and N523, obtained from H. G. Wittmann (see ref. 3)) for their susceptibility to transfection by PCRI DNA, a chimaeric plasmid obtained by inserting a fragment of PSC105 DNA, containing the kanamycin resistance marker, into colE1 DNA[4] and deleting one of the two *Eco*RI restriction sites[5].

E. coli N543 (a streptomycin-dependent derivative of *E. coli* AB 774, *rec*$^+$ *F*$^-$ *lac*$^-$ *xyl*$^-$ *gal*$^-$ *thi*$^-$ *arg*$^-$ *his*$^-$ *ile*$^-$, derived from *E. coli* K) was found to be very suitable for transfection; about 5×10^5 transfectants per μg of PCRI DNA were obtained under transfection conditions essentially as described by Mandel and Higa[6]. This strain compares favourably with the commonly used recipient *E. coli* HB101 (obtained from H. Boyer) which gave values of 1.8×10^5 transfectants per μg (Table 1). Since the streptomycin-dependent strain is *rec*$^+$ and contains the K restriction and modification system, it is as such not recommended for the cloning of chimaeric DNA. A clone of PCRI-containing *E. coli* N543 (*E. coli* N543 (PCRI)) was isolated and found to be *lac*$^-$, auxotrophic, resistant to kanamycin to at least 1,000 μg ml^{-1} and absolutely dependent on streptomycin for colony formation on agar plates. Half-maximal growth rate in tryptone medium required about 150 μg ml^{-1} and maximal rate 500 μg ml^{-1} of streptomycin. The level of streptomycin-independent revertants in stocks prepared from clones was about 1 in 10^9. Bacteria grown in streptomycin-containing tryptone broth continued to grow for some generations even after removal of the antibiotic, presumably until streptomycin-saturated ribosomes were diluted out of the bacteria.

Table 1 Transfection of various strains of *E. coli* by PCRI DNA

PCRI DNA from untreated bacteria	*E.coli* HB101 Colonies/ aliquot	*E.coli* HB101 Colonies/ µg DNA	*E coli* N543 Colonies/ aliquot	*E coli* N543 Colonies/ µg DNA
0 ng	0	—	0	—
5 ng	101	1.2×10^5	516	5.9×10^5
20 ng	630	$1\ 8\times10^5$	1,720	4.9×10^5
PCRI DNA from bacteria killed with diethylpyrocarbonate				
0 ng	0	—	0	—
5 ng	83	0.95×10^5	405	4.7×10^5
20 ng	623	1.8×10^5	1,760	5.1×10^5

The procedure is based on that of Mandel and Higa[6]. *E.coli* HB101 and *E.coli* N543 were grown in tryptone medium to an A_{650} (Beckman DB) of about 1. In the case of the strain N543 the medium contained 200 µg ml^{-1} of streptomycin sulphate. The bacteria were collected by centrifugation for 15 min at 3,000 r.p.m. in a Sorvall HB4 rotor, resuspended in 0.5 vol of 0.05 M $CaCl_2$ and kept for 15 min at 0 °C. The bacteria were then recentrifuged and resuspended in 0.3 vol (based on the original volume) of 0 05 M $CaCl_2$ The viable cell count was 5×10^8 ml^{-1} for *E coli* HB101 and 10^9 ml^{-1} for *E.coli* N543. To 50 µl of PCRI DNA solution (in 10 mM Tris-HCl (*p*H 7.5)–10 mM $CaCl_2$-10 mM $MgCl_2$) 100 µl of the bacterial suspension were added. The suspension was incubated for 15 min at 0 °C, 2 min at 37 °C and 10 min at room temperature 1 ml of tryptone medium (containing 200 µg ml^{-1} of streptomycin in the case of *E coli* N543) was added and incubation was continued for 1 h at 37 °C. 0.2 ml of each sample was then spread on McConkey agar containing 100 µg ml^{-1} of kanamycin (and 200 µg ml^{-1} of streptomycin in the case of *E. coli* N543). Tryptose medium contained per litre: Bacto tryptone, 10 g; yeast extract (Difco) 1 g; glucose, 1 g; NaCl, 8 g; $CaCl_2 . 2H_2O$, 0.294 g

Table 2 Effect of diethylpyrocarbonate or chloroform on the viability of bacterial cultures

Treatment	Time of exposure	Viability after treatment		
Diethylpyrocarbonate		at *p*H 5.5	at *p*H 6.5	at *p*H 7.5
	None	1.3×10^8	1.7×10^8	1.8×10^8
	15 s	0	0	ND
	30 s	0	0	<10
	45 s	0	0	0
	60 s	0	0	0
Chloroform		at *p*H 6 2		
	None	3.4×10^8		
	60 s	500		
	90 s	67		
	120 s	0		
	240 s	0		

E.coli N543 (PCRI) was prepared by transfecting *E.coli* N543 with PCRI DNA as described in the legend to Table 1. The bacteria were grown in 100 ml of tryptone medium to an A_{650} of 0.8–0.9 on the Beckman DB spectrophotometer. At this point the *p*H of the culture had fallen from 7.2 to 6.2. Samples were adjusted to the *p*H indicated and 0.3 vol% of diethylpyrocarbonate or 1 vol% of chloroform were added with vigorous stirring at 37 °C At the times indicated 1-ml aliquots were removed and diluted with 9 ml of chilled tryptone medium adjusted to *p*H 8 with NH_4OH. Each sample was diluted where appropriate, and a 1-ml sample was immediately filtered through a Millipore HAWP 0.45 µm filter (25 mm diameter). The filters were placed face upward on McConkey agar containing 200 µg ml^{-1} streptomycin and 100 µg ml^{-1} kanamycin and incubated at 37 °C overnight.

A late log phase culture of *E. coli* N543 (PCRI) was vigorously stirred with 1 vol% of chloroform or 0.3 vol% of diethylpyrocarbonate at 37 °C. At appropriate intervals 1-ml samples were diluted into 9 ml of chilled tryptone medium, and the viable bacteria were immediately determined. No surviving bacteria could be detected in the equivalent of 1 ml of the original culture after 120 s of treatment with chloroform or after 15 s of treatment with diethylpyrocarbonate (Table 2). Diethylpyrocarbonate was equally effective at an initial *p*H of 5.5, 6.5 or 7.5.

For purification of plasmid DNA from untreated and killed *E. coli*, a log phase culture of chloramphenicol-treated ^{32}P-labelled *E. coli* N543 (PCRI) was divided into two. One part was collected immediately, whereas the other was vigorously shaken with diethylpyrocarbonate for 5 min at 37 °C. NH_4OH was added to *p*H 7.5 to accelerate the decomposition of excess reagent after which the cells were collected. Both bacterial pellets were then suspended, lysed with Triton X-100 and the plasmid DNA was purified by a modification of the method of Katz *et al.*[7]. The yields and the specific infectivities of PCRI DNA (form I) were essentially the same for both samples (Table 3). Treatment with diethylpyrocarbonate thus affected neither the extraction nor the biological activity of the plasmid DNA. Similar results were obtained by the purification method of Guerry *et al.*[8] which we found more convenient for the preparation of large quantities of plasmid DNA. Treatment of *E. coli* N543 (PCRI) with diethylpyrocarbonate for 10 min or longer occasionally rendered the bacteria less susceptible to lysis with Triton X-100; however, lysis with 1% sodium dodecyl sulphate, as used in the method of Guerry *et al.*[8] was always successful.

Bacteria killed with chloroform were also perfectly suitable starting material, both with regard to yield and specific infectivity of plasmid DNA (data not shown).

The simple killing step introduced before collection

Table 3 Purification of PCRI DNA from native or diethylpyrocarbonate-killed *E.coli* N543 (PRCI)

Yield	*a*, From native *E.coli*		*b*, From diethylpyrocarbonate-killed *E.coli*	
	A_{260} units	Total c.p.m.	A_{260} units	Total radio activity (c.p.m.)
Step 1 (Crude DNA)	ND	1.4×10^9	ND	1.5×10^9
Step 2 (after CsCl density gradient)	0.38	2.8×10^6	0.42	3.0×10^6
Step 3 (after sucrose density gradient)	0.26	1.6×10^6	0.36	1.7×10^6

A 2-l flask containing 0.5 l of low-phosphate tryptone medium (final concentration of inorganic phosphate, 0.25 mM) with 200 μg ml^{-1} of streptomycin was inoculated with *E.coli* N543 (PCRI) and 1 h later 10 mCi of carrier-free ^{32}P-phosphate were added. At an A_{650} (Beckman DB) of about 0.8, chloramphenicol was added to a final concentration of 0.17 mg ml^{-1} and incubation continued for 14 h at 37 °C. The culture was divided into two 250-ml portions. Portion *a* was chilled immediately. Diethylpyrocarbonate (0.75 ml) were added to portion *b*. After 5 min at 37 °C two drops of 1% phenol red and concentrated NH_4OH were added until the indicator turned red and the culture was chilled. A 1-ml aliquot of this suspension was diluted with 10 ml of medium, the bacteria were collected by centrifugation, resuspended in 0.2 ml tryptone medium and spread on a streptomycin-containing (200 μg ml^{-1}) McConkey agar. No colonies were observed after 24 h. The bacteria were collected by centrifugation, and plasmid DNA was extracted essentially by the method of Katz *et al.*[7]. The bacteria were suspended in 50 ml of 10 mM Tris-HCl (*p*H 8.0), 1 mM EDTA, centrifuged and resuspended in 10 ml of 25% sucrose, 50 mM Tris-HCl (*p*H 8.5). Lysozyme (12.5 mg) was added and after 5 min at 0 °C, EDTA (*p*H 8.5) was added to 50 mM. After 5 min at 0 °C 10 ml of a solution containing 0.1% Triton, 0.53 M EDTA, 0.05 M Tris-HCl (*p*H 8.5) were added. After 1 h at 0 °C the viscous lysates were cleared by centrifugation and extracted with one volume of phenol–cresol–hydroxyquinoline (500:70:0.5 v/v/w) (step 1 preparation). The nucleic acids were precipitated with ethanol, redissolved and centrifuged to equilibrium in a CsCl gradient containing ethidium bromide. The lower stained band was collected, extracted 3 times with 1 vol of isoamylalcohol, dialysed and precipitated with ethanol (step 2 preparation). The preparation was centrifuged through a 4.5-ml 5–23% sucrose gradient in 50 mM Tris-HCl (*p*H 7.5) for 90 min at 58,000 r.p.m. and 15 °C in the Spinco SW60 rotor. The single peak of ^{32}P-PCRI DNA (step 3 preparation) was located by Cerenkov counting. Analysis of the DNA by agarose gel electrophoresis showed the exclusive presence of form I PCRI DNA in both samples *a* and *b*.

eliminates one of the major sources of environmental contamination in work with potentially pathogenic *E. coli* and should greatly simplify the physical containment requirements which might otherwise be necessary for the processing of larger quantities of bacterial cells. The use of streptomycin-dependent strains of *E. coli* should further reduce the probability of unwanted propagation of plasmid-containing *E. coli.*

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Phosphorylation of yeast RNA polymerases

DNA-DEPENDENT RNA polymerase activities in eukaryotic cells change during developmental[1,2] and physiological transitions[3–8]. Some changes occur rapidly and in the absence of protein synthesis. They presumably involve modulation of the activity of pre-existing RNA polymerase molecules rather than alterations of the rate of synthesis or degradation of these molecules. This modulation of polymerase activity could be a consequence of covalent modification of enzyme subunits, the rapid turnover of a protein factor required for catalysis, alteration of the template properties of the chromatin or some combination of these. The first possibility is attractive. The activity of many enzymes is controlled in this manner[9]. Cycles of phosphorylation and dephosphorylation regulate the activity of the enzymes involved in glycogen metabolism (reviewed in refs 9 and 10). Lee *et al.*[11] observed an increase in the specific activity of Rous sarcoma virus reverse transcriptase on incubation with protein kinase and ATP. Phosphoryla- of β and β′ subunits of *E. coli* RNA polymerase on infection by bacteriophage T7 has been implicated in the control of early transcription of T7 DNA[12]. Several groups[5,13,14] have proposed and presented preliminary evidence that phosphorylation of RNA polymerase is involved in regulation of polymerase activity in eukaryotes. Jungmann *et al.*[5] and Martelo and Hirsch[13] observed enhanced RNA polymerase activity when partially purified protein kinase, RNA polymerase and ATP were mixed. They observed the phosphorylation of proteins in their preparations and attributed the increase in polymerase activity to phosphorylation of the enzyme. They did not demonstrate phosphorylation of RNA polymerase polypeptides, and since the observed increase in RNA polymerase activity could also be a consequence of phosphorylation of contaminating acidic nuclear proteins which can stimulate polymerase activity[15–17], the cause of the enhanced polymerase activity is obscure. We report here the isolation of phosphorylated RNA polymerase I from yeast cells and the identification of the enzyme polypeptides phosphorylated *in vivo*. The same pattern of phosphorylation was obtained when highly purified RNA polymerase I was incubated with a yeast protein kinase preparation.

Yeast cells were grown in complete medium in the presence of ^{32}P-phosphate and collected in the late log phase of growth. RNA polymerase I was purified partially, mixed with unlabelled RNA polymerase I and subjected to sucrose gradient centrifugation. A significant fraction of the phosphorylated proteins sedimented with the enzymatic activity of the added RNA polymerase (Fig. 1*a*). The possible relationship of the phosphorylated polypeptides to the putative RNA polymerase I subunits was determined by resolution of the protein from the sucrose gradient fractions by polyacrylamide gel electrophoresis and subsequent autoradiography of the dried gel. Phosphorylated polypeptides migrated together with five of eleven polymerase I polypeptides (Fig. 1*b* and *c*). The 185,000, 44,000 35,000 and 24,000-dalton polypeptides were relatively more labelled than the 20,000-dalton polypeptide. There was also a small amount of radioactivity which migrated with the dye-front and may have been RNA. These data indicate that RNA polymerase is phosphorylated *in vivo* and is a phosphoprotein as isolated by common procedures.

Purified yeast RNA polymerases can be phosphorylated

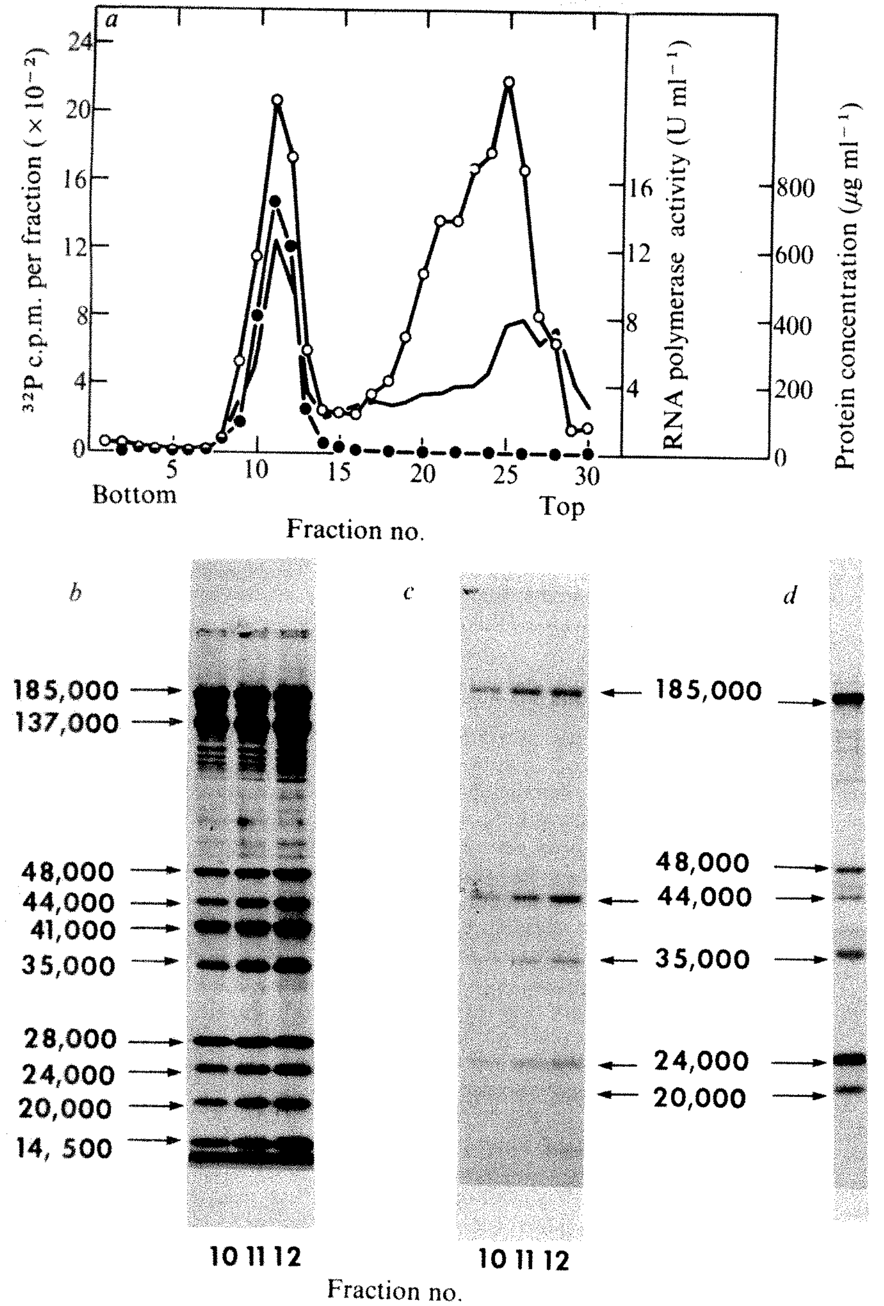

Fig. 1 Phosphorylation of yeast RNA polymerase I. ^{32}P-labelled RNA polymerase I was isolated from a prototrophic tetraploid strain of *Saccharomyces cerevisiae* (provided by Dr L. Hartwell) grown in complete medium (YEPD)[18] containing ^{32}P-phosphate (5 μCi ml^{-1}). Cells (2 l) were collected in the late-log phase of growth (30 g, wet weight) and spheroplasts were prepared by the two-step procedure of Cabib[19] as described by Wintersberger *et al.*[20]. RNA polymerase I was extracted in the presence of 1 mM ATP and was purified to fraction 3 by a micro-adaptation of Valenzuela *et al.*[21]. The ^{32}P-labelled enzyme fraction was mixed with unlabelled polymerase I (fraction 4)[21] and protein was precipitated by dialysis against a saturated ammonium sulphate solution. The precipitate was collected by centrifugation (10,000*g*, 20 min) and dissolved in 15% glycerol, 0.2 M KCl, 0.1 mM EDTA, 6 mM 2-mercaptoethanol, 50 mM Tris-HCl, *p*H 7.9. The RNA polymerase I was further purified by centrifugation in a 5–20% (w/v) sucrose gradient in 0.5 M KCl, 15% glycerol, 0.1 mM EDTA, 6 mM 2-mercaptoethanol, 50 mM Tris-HCl, *p*H 7.9 (SW56 rotor, 56,000 r.p.m., 15 h, 4 °C). Fractions were collected by tube puncture. *a*, Sucrose gradient profile of RNA polymerase I phosphorylated *in vivo*. RNA polymerase activity and protein concentration of each fraction were determined as before[21]. An aliquot of each gradient fraction was spotted on to a glass fibre filter (GF/C, Whatman). Filters were washed in 10% trichloroacetic acid (TCA). Contaminating ^{32}P-labelled nucleic acid was hydrolysed by boiling the filters in 5% TCA for 15 min. The filters were then washed twice more in 5% TCA, then in ethanol and dried. The ^{32}P-labelled protein was counted in Omnifluor (New England Nuclear)–toluene scintillant. *b*, Resolution of polypeptides by sodium dodecyl sulphate (SDS)–acrylamide gel electrophoresis of *in vivo* ^{32}P-labelled RNA polymerase. The polypeptides in each sucrose gradient fraction were resolved by 10% acrylamide slab–gel electrophoresis in the presence of 0.1% SDS[21] and stained with Coomassie blue. Buffers and solutions were prepared according to Laemmli[22]. The putative subunits of RNA polymerase I and assigned molecular weights are indicated by the arrows. *c*, Autoradiogram of RNA polymerase polypeptides labelled *in vivo* with ^{32}P. The stained gel prepared in (*b*) was dried and the ^{32}P-polypeptides were located by autoradiography. *d*, *In vitro* phosphorylation of yeast RNA polymerase I subunits. The standard reaction mixture contained in 0.050 ml: 10 μg of purified RNA polymerase I (21), 0.2 U of yeast protein kinase (1 U of protein kinase activity defined as 1 nmol of ^{32}P transferred from ^{32}P-ATP to 200 μg of phosvitin in 10 min at 30 °C), 60 mM Tris-HCl, *p*H 7.9, 5 mM $MgCl_2$, 6 mM NaF, 90 mM KCl, 5 mM 2-mercaptoethanol, 0.010 mM ^{32}P-ATP (specific activity, 1,000 d.p.m. pmol^{-1}) and 7.5% glycerol. After incubation for 15 min at 30 °C, the reaction was stopped by the addition of 0.005 ml of 5 mM ATP and subsequent transfer to 0 °C. Phosphorylated polymerase was precipitated from 10% TCA and the labelled polypeptides were identified after SDS–acrylamide gel electrophoresis and autoradiography as described above. No phosphorylation was observed in the absence of either polymerase or protein kinase.

in vitro with a protein kinase which is purified together with RNA polymerase I but is resolved on sucrose gradient centrifugation. Whether this kinase is identical to those reported in yeast is unknown[23,24]. This enzyme uses either ATP or GTP as a phosphate donor, is not stimulated by cyclic AMP and will phosphorylate both acidic and basic phosphoprotein acceptors (G. I. B., unpublished results). The incubation of purified polymerase I and kinase resulted in the phosphorylation of the five polypeptides phosphorylated *in vivo* and, in addition, the 48,000-dalton polypeptide (Fig. 1*d*). The 185,000 and 24,000-dalton polypeptides were phosphorylated to a greater degree than the other polypeptides. The protein kinase also phosphorylated other molecules in the polymerase preparation. These phosphorylated non-polymerase polypeptides did not migrate together with stained polypeptides in the gel and it is not known whether they are a consequence of proteolysis of enzyme polypeptides or are contaminants of the polymerase preparation. Enzyme polypeptides of purified yeast RNA polymerases II and III were also phosphorylated by the protein kinase preparation (data not shown). The 33,500 and 24,000-dalton polypeptides of polymerase II and the 53,000, 24,000 and 20,000-dalton polypeptides of polymerase III were phosphorylated. (The isolation and subunit composition of polymerases II and III have been reported elsewhere[25,26].) Three of the phosphorylated polymerase I polypeptides (molecular weight 185,000, 44,000 and 35,000) are unique to polymerase I. Two-dimensional polyacrylamide gel electrophoresis showed the 24,000-dalton polypeptide to be common to RNA polymerases I, II and III; the 20,000-dalton polypeptide seems common to polymerases I and III (P.V., unpublished). The phosphorylation of the 24,000-

dalton polypeptide of polymerases I, II and III and the 20,000-dalton polypeptide of polymerases I and III supports the contention that these are common subunits between the respective enzymes. Four of the phosphorylated polymerase I polypeptides (molecular weight 48,000 44,000, 35,000 and 24,000) are readily dissociated from the enzyme and seem to be important for activity. Polymerase I has been isolated that lacks the 48,000 and 44,000-dalton polypeptides[26] or the 48,000 and 35,000-dalton polypeptides[27] and has reduced enzymatic activity on double-stranded DNA templates. Polymerase I lacking the 24,000-dalton polypeptide has also been isolated and is inactive on single- and double-stranded DNA and poly d(A-T) templates (P. V. and G. I. B., unpublished results).

In the phosphorylation experiments with purified polymerase I and protein kinase *in vitro*, only 0.5–1.0 mol of phosphate was bound to each mole of polymerase in optimal conditions. Since the data of the *in vivo* experiments indicate that there are at least five phosphorylation sites per enzyme, we presume that the purified polymerase is partially phosphorylated as isolated. The polypeptides of the purified polymerase molecules are phosphorylated to different extents in the *in vivo* and *in vitro* experiments. For example, the 44,000-dalton polypeptide is labelled more *in vivo* than *in vitro* while the reverse is found with the 24,000 and 20,000-dalton polypeptides. Thus there may be an equilibrium in the cell between phosphorylated and non-phosphorylated polymerase polypeptides. We observed that the 48,000-dalton peptide is phosphorylated only *in vitro*. One explanation for this result is that the enzyme molecule exists as a complex within the cell, such that the 48,000-dalton polypeptide is not accessible to protein kinase.

By modulating the charge density at a specific site in a polypeptide, phosphorylation and dephosphorylation could control the extent of interaction between enzyme polypeptides or between the enzyme and other molecules. In this way, phosphorylation of RNA polymerase could have a direct effect on transcription by altering the specific activity of the enzyme. It could also have an indirect effect on activity by modulating the association of polymerase with regulatory molecules or chromatin. Alternatively, phosphorylation may alter the turnover of the enzyme. We have begun to test the effects of phosphorylation on these aspects of polymerase function. In preliminary experiments, incubation of purified polymerase I with protein kinase and ATP or with bacterial alkaline phosphatase produced no effects on enzyme activity. These experiments are inconclusive so far because the isolated polymerase I is phosphorylated and the phosphatase may not remove the phosphate residues. We shall test the effects of phosphorylation on activity in standard assay conditions as well as the transcription of ribosomal genes in more physiological conditions, to elucidate the relationship between the phosphorylation of polymerase I and transcription.

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Association of RNase H activity with yeast RNA polymerase A

EUKARYOTIC RNA polymerases were isolated as large multimeric protein complexes containing two high molecular weight subunits and a collection of smaller polypeptide chains[1–3]. This structural complexity suggests a multifunctional system, organised around a core enzyme combined with specificity determinants and possibly other proteins involved in regulation of chromatin activity. The purpose of this report is to describe the association of a ribonuclease H activity with yeast RNA polymerase A. This nuclease activity, which specifically degrades RNA–DNA hybrids, is inseparable from the polymerase by various fractionation procedures.

In the experiment shown in Fig. 1*a*, highly purified RNA polymerase A from yeast cells was subjected to phosphocellulose chromatography. The fractions were tested for RNA polymerasing activity as well as for their ability to degrade the homopolymer ^{3}H–(rA)$_n$ or the hybrid duplex ^{3}H–(rA)$_n$·(dT)$_n$. As previously reported[4], RNA polymerase A was separated into two enzymatic fractions, RNA polymerase A*, that lacked two polypeptide chains, and the complete enzyme A. A nuclease activity that degraded the (rA)$_n$ moiety of the hybrid into acid-soluble material was also revealed. But no degradation of the ribohomopolymer (rA)$_n$ occurred. A coincident distribution of polymerase and nuclease activities was found at the level of the two protein peaks (Fig. 1*a*). When RNA polymerase A from phosphocellulose was subjected to zone sedimentation through a 10 to 30% glycerol gradient, the nuclease cosedimented with the polymerase activity (Fig. 1*b*). The two peaks A and A*, from phosphocellulose, were further chromatographed separately on DEAE–cellulose. Again, RNA polymerase A (Fig. 1*c*), as well as A* (result not shown), were eluted in a sharp protein peak, simultaneously with the (rA)$_n$·(dT)$_n$ degrading activity. Furthermore, the nuclease, when subjected to electrophoresis on polyacrylamide gel under non-denaturing conditions, also had the same electrophoretic mobility as RNA polymerase. Again the nuclease was recovered at the level of the protein bands corresponding to RNA polymerase A and A* (ref. 4) (result not shown). From the above observations, since the nuclease activity always copurified with RNA polymerase during ion exchange chromatography, zone sedimentation and electrophoresis, it was concluded that the nuclease was physically associated with the RNA polymerase molecule.

As already seen in Fig. 1*a* the presence of the hybridised $(dT)_n$ strand was required for degradation of the $(rA)_n$ polymer. Likewise, removal of the $(dT)_n$ moiety of the hybrid by DNase prevented the degradation of the ribopolymer strand (Table 1). The polymerase-associated nuclease activity therefore had the properties of a ribonuclease H (ref. 5). Degradation of hybridised RNA was not restricted to the synthetic homopolymer duplex $(rA)_n{\cdot}(dT)_n$. ^{3}H-RNA–T7 DNA hybrid, synthesised with *Escherichia coli* RNA polymerase, was also used as substrate with similar efficiency. On the other hand, the enzyme was completely free of DNase activity, as tested with ^{3}H-T7 DNA by sedimentation in alkaline sucrose gradient.

It should be stressed that the ratio of RNase H compared with polymerase activity, as seen in Fig. 1*a*, is misleading, because the degradation reaction was not linear with time. The reaction tended to plateau at about 40 to 50% completion. Kinetic measurements, in fact, indicated that, as far as RNase H was concerned, the specific activity of A* was four times lower than that of the complete enzyme A. This suggested that the two polypeptides missing in A* are in some way involved in the degradation process. Experiments with yeast RNA polymerase B did not reveal the presence of similar nuclease activity in this enzyme. Therefore, it is unlikely that the three common subunits of A and B enzymes[3] are implicated.

The requirements of the nuclease are summarised in Table 1. In the above experiments, the activity was assayed at *p*H 8 (Fig. 1*a* and *b*) or *p*H 6.5 (Fig. 1*c*). In fact, $(rA)_n{\cdot}(dT)_n$ degradation occurred within a broad range of *p*H, between *p*H 6 and *p*H 8. It required the presence of divalent cations, either Mg^{2+} (5 mM), or preferably Mn^{2+} (1 mM), for optimal activity, and was sensitive to the ionic strength of the reaction mixture (Table 1). In the presence of 100 mM KCl, the nuclease retained only 27% of its optimal activity. Among other compounds, inorganic pyrophosphate (0.1 mM), phosphate (0.1 mM) or α-amanitin, were neither stimulatory nor inhibitory.

Since the requirements for RNase H activity associated with the polymerase were optimally met by the conditions used for transcription, the question arose whether the nuclease activity was expressed during RNA synthesis. With $(rA)_n{\cdot}(dT)_n$ as template primer, ATP can be incorporated by yeast RA polymerase A, possibly through *de novo* chain initiation or by elongation of pre-existing RNA chains (result not shown). In a challenge experiment, $(rA)_n{\cdot}(dT)_n$ degradation was followed in the presence of added ATP or with a different nucleoside triphosphate. The results are summarised in Table 1. With 0.5 mM ATP, in conditions where RNA synthesis took place, RNase H activity was depressed to an insignificant level. By contrast,

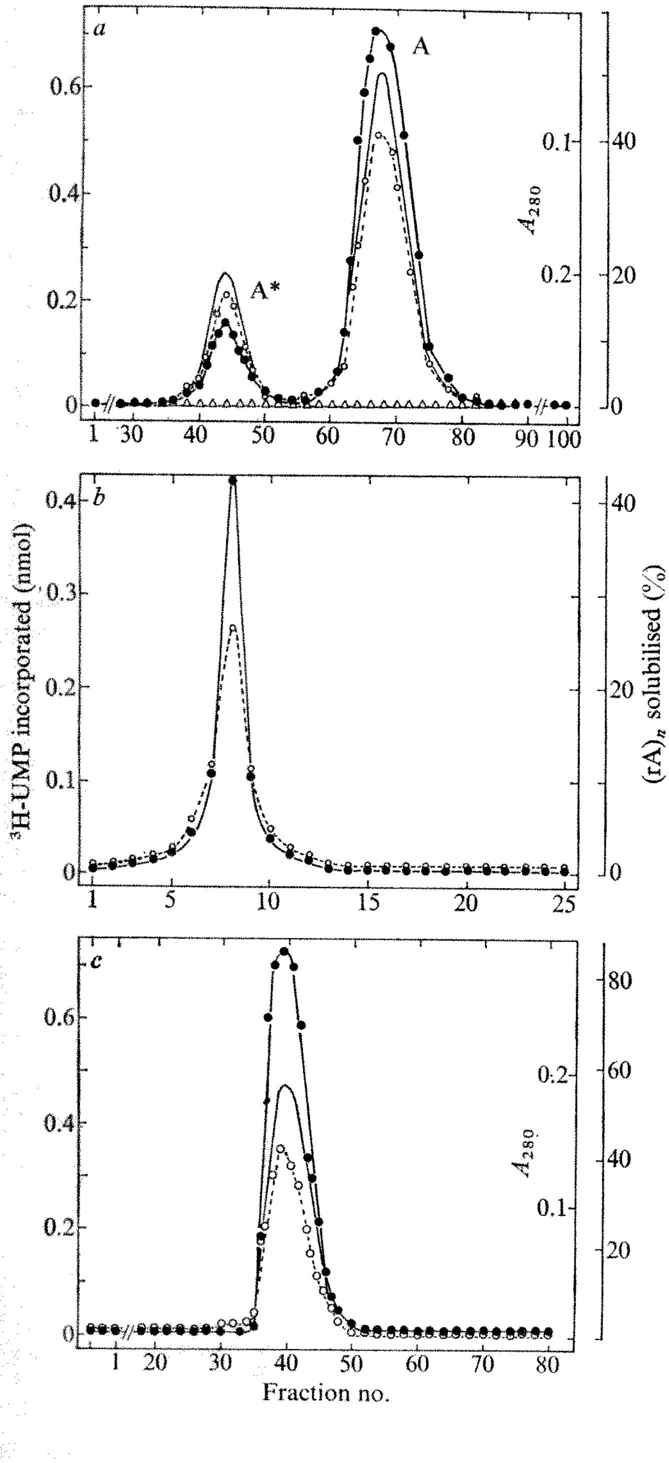

Fig. 1 Copurification of RNA polymerase and RNase H activities. *a*, Highly purified RNA polymerase A (5.8 mg of protein, glycerol gradient step) was chromatographed on phosphocellulose as previously described[4], and the fractions (1 ml) were analysed for absorbance (—), RNA polymerase (●), RNase H (○) and $(rA)_n$ degrading (△) activities; *b*, RNA polymerase A from the phosphocellulose column (50 µg of protein, peak A) was loaded on top of 4.8 ml of a 10–30% glycerol gradient, in a buffer containing 0.02 M Tris-HCl *p*H 8, 0.5 mM EDTA, 0.01 M 2-mercaptoethanol, 0.05 M ammonium sulphate. The gradient was centrifuged for 18 h, at 40,000 r.p.m., in a SW65 rotor, at 2 °C. Fractions (0.2 ml) were collected from the bottom of the tube and assayed for RNA polymerase (●) and RNase H (○) activities. *c*, RNA polymerase A (3 mg of protein, peak A from phosphocellulose) was dialysed for 1.5 h at 4 °C against a buffer containing 0.02 M. Tris-HCl, *p*H 8, 0.5 mM EDTA, 0.01 M 2-mercaptoethanol, 0.05 M ammonium sulphate and 30% glycerol (v/v), and applied to a DEAE–cellulose column ($0.5\ cm^2 \times 12$ cm) equilibrated with the same buffer containing 10% glycerol (v/v). The column was developed with 100 ml of a linear gradient from 0.05 M to 0.4 M ammonium sulphate in the above buffer. Fractions (1.4 ml) were collected and assayed for absorbance, (—), RNA polymerase (●), and RNase H (○) activities. RNA polymerase was assayed in 0.1 ml mixtures containing 0.06 M Tris-HCl, *p*H 8, 1 mM dithiothreitol, 5 mM $MgCl_2$, 1 mM each of ATP and ^{3}H-UPT (25 c.p.m. pmol^{-1}), 3 µg of $d(A\text{-}T)_n$ and 10 µl from the various fractions. After 30 min incubation at 30 °C, radioactivity of acid-precipitable material was measured. RNase H activity was assayed in 0.1 ml mixtures containing ^{3}H-$(rA)_n{\cdot}(dT)_n$ as substrate (0.22 nmol of $(rA)_n$; 36 c.p.m. p mol^{-1} of polymerised nucleotide) 0.06 M Tris-HCl, *p*H 8.1 dithiothreitol, 5 mM $MgCl_2$ and 10 µl sample from the various fractions. In the case of the DEAE cellulose column, the assay was conducted at *p*H 6.5 with 0.025 M Tris-maleate buffer and with 0.05 mM $MgCl_2$. After 1 h incubation at 37 °C, 100 µg serum albumin (50 µl) were added as carrier, followed by 15 µl of 50% trichloroacetic acid; the precipitate was discarded by low speed centrifugation, and radioactivity of acid-soluble material was measured. The results are expressed as percentage of radioactivity released from the hybrid, relative to the input. $(rA)_n$-degrading activity was assayed in the above conditions, using the same amount of non-hybridised ^{3}H-$(rA)_n$ as substrate.

CTP or GTP, which were non-complementary nucleotides in this system, did not significantly affect degradation of the $(rA)_n$ strand. Only UTP, which was complementary to the ribopolymer strand, was somewhat inhibitory, although to a lesser extent than ATP. To demonstrate in addition that inhibition caused by ATP was linked to the occurrence of the polymerisation reaction, the effect of α-β methylene ATP (0.5 mM) was investigated. This analogue of ATP cannot be used for chain elongation, but can support pyrophosphate exchange[6], as initiator nucleotide, in the presence of $d(A\text{-}T)_n$ and UTP (ref. 7). As expected, this analogue did not influence the RNase H activity. The absence of degradation during RNA synthesis probably reflected the processivity of the polymerisation reaction. This also indicated that the nuclease remained associated to the polymerase molecule during transcription.

Table 1 Requirements for $(rA)_n{\cdot}(dT)_n$ degradation

Addition or omission	Acid-solubilised radioactivity (c.p.m.)	% Hybrid degradation
Complete system	4,100	25.5
$-MgCl_2 + MnCl_2$ (1 mM)	7,800	49
$-MgCl_2$	200	1
+10 μg DNase at zero time	213	1
+20 μg α-amanitin	3,880	24.5
+KCl (50 mM)	1,942	12
+KCl (100 mM)	1,130	7
$+PP_i$ (0.1 mM)	4,120	25.5
$+P_i$ (0.1 mM)	4,100	25.5
+ATP (0.5 mM)	197	1
+UTP (0.5 mM)	2,480	15.5
+GTP (0.5 mM)	3,340	21
+CTP (0.5 mM)	3,600	22.5
+α-β methylene ATP (0.5 mM)	3,500	22

RNA polymerase A (1 μg, from the DEAE-cellulose column shown in Fig. 1) was incubated, for 1 h at 37 °C, in 0.2 ml of a mixture containing $^3H\text{-}(rA)_n{\cdot}(dT)_n$ (448 pmol of polymerised ribonucleotides; 36 c.p.m. pmol^{-1}), 0.06 M Tris-HCl, *p*H 8.1, 5 mM $MgCl_2$ and 1 mM dithiothreitol. Radioactivity of acid-soluble material was measured as described in Fig. 1.

Nuclease activities were found physically associated with bacterial and phage-coded DNA-dependent DNA polymerases[8]. Mölling *et al.*[9], also discovered the RNase H activity of viral reverse transcriptase. This, however, is the first time that a nuclease activity (clearly distinct from the reversal of polymerisation through pyrophosphorolysis) is found associated with a DNA-dependent RNA polymerase. The existence of this RNA polymerase-associated RNase H has significant implications in the consideration of the rôle of DNA–RNA hybrids in transcription. It is possible that RNA–DNA hybrids are involved in chromatin structure, perhaps regulating its template activity. Since yeast RNA polymerases seem to prefer unpaired DNA structures to initiate[10,11], transient RNA–DNA hybrids could also be formed at the initiation sites[11] and removed by the RNase H. Among other possibilities one could also consider a proof reading function for the polymerase. Ascribing a definitive physiological rôle to this nuclease activity will finally depend on genetic evidence.

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Control of 5S RNA synthesis in *Xenopus laevis*

PREVIOUS reports[1–3] have shown that somatic cells of *Xenopus laevis* synthesise a 5S RNA which differs in sequence from 5S RNA synthesised by oocytes. In none of these reports is there any indication of ovary-type sequences being synthesised by somatic cells. Moreover, although the oocyte 5S sequences are heterogeneous, the somatic sequence is essentially homogeneous. We report here that *X. laevis* liver cells labelled in organ culture do not detectably synthesise ovary-type 5S RNA sequences but find that a transformed tissue culture cell line, derived initially from somatic kidney cells, synthesises significant (10–20%) amounts of ovary-type 5S RNA sequences. Taken together with the observation that some 5S genes are translocated to a novel site adjacent to the nucleolar organiser in these cells[4] this result is important for an understanding of the nature of differential control of 5S gene transcription in *X. laevis*.

Total 5S RNA labelled with $^{32}PO_4$ was prepared from either *Xenopus* liver cells or a permanent tissue culture cell line as described in the legend to Fig. 1. Oligonucleotide maps from complete RNase T_1 and RNase A digests of 5S RNA were made as described previously[3] and the molar yields estimated. In liver, yields of radioactivity in regions of the chromatogram corresponding to the expected positions of oocyte-specific oligonucleotides were also counted to set an upper limit on the amount of oocyte type 5S sequence transcribed. In all cases blank areas of chromatogram were counted to provide background radioactivity estimates which were substracted from the count rates of oligonucleotides. The results (Table 1) show that the three major oocyte-specific T_1 products (X_1, X_2 and X_3) and the one specific RNase A product (X_1) are all present with yields of 10–20% in 5S RNA from tissue culture cells but are not detectable above background in 5S RNA

Table 1 Molar yields of oligonucleotides from RNase T_1 and RNase A digests of 5S RNA from liver cells, tissue culture cells and oocytes of *X. laevis*

Nucleotide spot number*	Liver	Cell type TC1†	TC2†	Oocyte*
RNase T_1 products				
14 CCCG	1.03	0.83	0.92	0.09
17 ACCG	1.09	0.96	0.97	0.50
24 CCUG	1.83	2.47	2.23	2.90
51a CCAAG	1.07	0.77	0.90	0.27
53 AUCUCG	1.91	1.85	1.89	0.98
52 UACUUG	0.93	0.78	0.81	0.07
X_1 AUCUCAG	0.01	0.23	0.10	0.70
X_2 UACCUG	0.01	0.19	0.13	1.06
X_3 AUACAG	0.01	0.18	0.08	0.72
RNase A products				
107 GGAAGC	1.12	0.76	0.91	0.18
X_1 AGAAGC	0.01	0.21	0.08	0.87

* Oocyte yields and spot numbers are those given in ref. 3.

† TC1 and TC2 refer respectively to tissue culture cells maintained by continuous passaging for 3 yr or in liquid nitrogen for 5 yr before analysis. Yields are the mean values for two determinations on two independent RNA preparations.

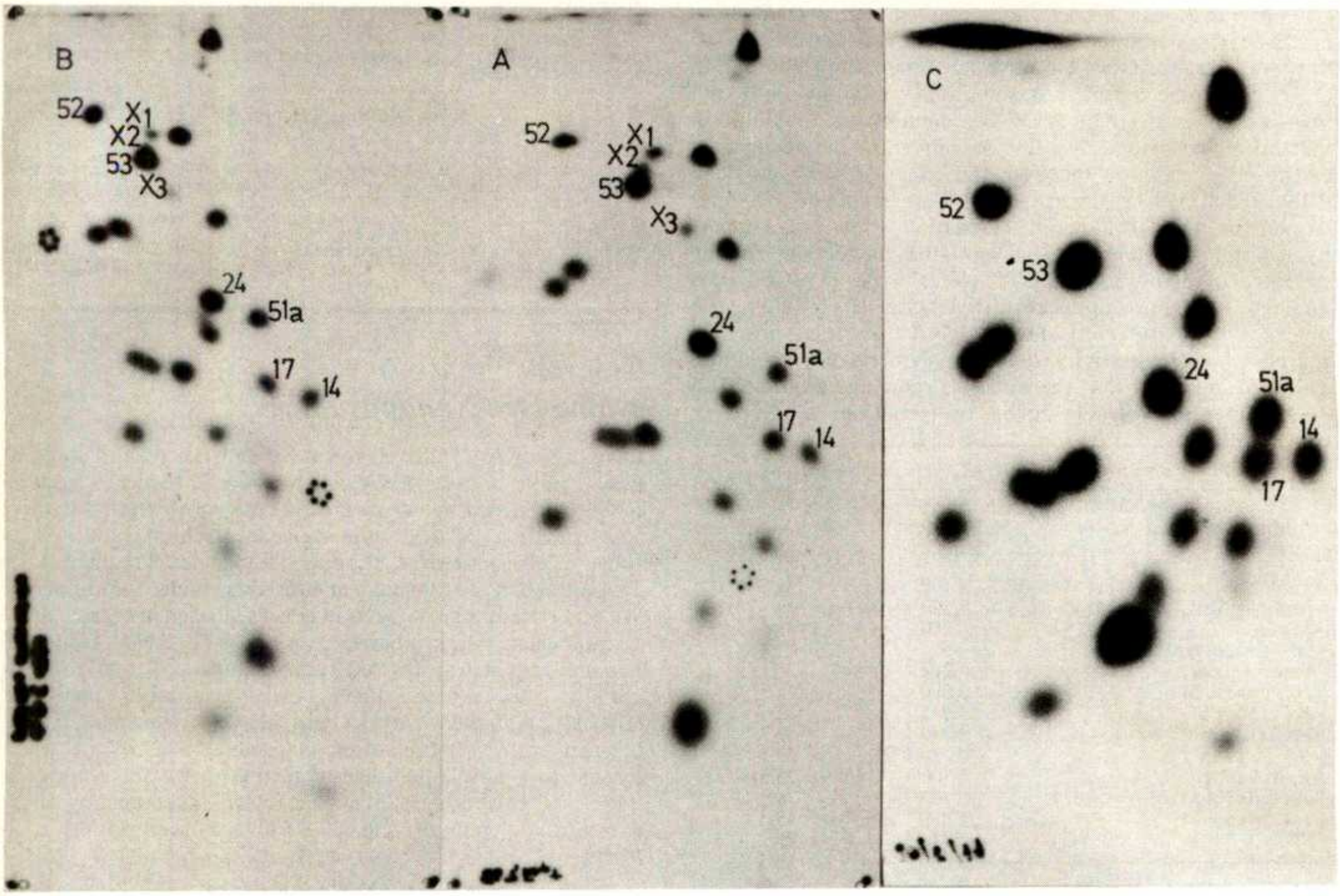

Fig. 1 Two-dimensional separation of RNase T_1 digestion products from ^{32}P 5S RNA labelled by tissue culture cell line 1 (*A*), tissue culture cell line 2 (*B*), and liver cells maintained in organ culture (*C*). Both tissue culture cell lines were derived from that established by Dr K. A. Rafferty in 1968 from adult *X. laevis* kidney. Tissue culture cell line 1 had been continuously grown over 3 yr before analysis, whereas tissue culture cell line 2 was derived from an ampoule of cells stored in liquid nitrogen for 5 yr before analysis. Tissue culture cells were grown to one-third confluence in Eagle's MEM plus 10% foetal bovine serum and diluted by 20% with water. They were labelled with carrier-free ^{32}P-orthophosphate (0.2 mCi ml^{-1}) for 3 d in the same medium containing one-third normal phosphate. Livers were removed from immature animals and rinsed well to remove as much blood as possible. They were pressed through a sterile 1-mm steel mesh. The small lumps of tissue ($\approx$ 1-mm cubes) were washed 5 times with fresh culture medium to remove broken cells and cultured for 48 h with carrier-free ^{32}P-orthophosphate (0.2 mCi ml^{-1}) in Eagle's MEM modified as above and containing one-third normal phosphate. 5S RNA was extracted, purified, digested with T_1 RNase and chromatographed as described previously[3].

from liver cells. Further sequence analysis on these cell line products proved that they are identical with the sequences previously reported for oocyte oligonucleotides migrating to the same position on the chromatograms[3].

We interpret these results as indicating a breakdown in the tissue culture line of the rigorous repression of oocyte-type 5S RNA transcription normally seen in somatic cells. The *Xenopus* kidney cell line is a transformed heteroploid cell line which has been maintained in culture for many years and has undergone considerable karyotype evolution. Nevertheless, a sample of the same cell line which had been kept in liquid nitrogen for several (five) years before analysis also produced oocyte-type 5S RNA sequences indicating that the event giving rise to leakiness of oocyte 5S gene repression must have been an early event in the establishment of this line, and is a constant and heritable change in the expression of 5S genetic material.

Genes coding for 5S RNA are present in many copies (24,000) in the genome[5] and are localised in clusters at the ends of the long arms of most of the chromosomes in *X. laevis*[4]. In the normal karyotype, no 5S genes are detected in the region of the nucleolar organiser; however, *in situ* hybridisation of 5S cRNA to chromosomes of the same tissue culture cell line as used in these sequencing studies reveals significant 5S genetic material in this position. Morphologically, also, there seems to have been addition of chromosome material distal to the nucleolar organiser, presumably by translocation, in these cells. Since the *in situ* technique will not distinguish between oocyte and somatic genes, it is not certain that this translocation involves oocyte-type 5S genes. Although normal liver cells must differ in many other ways from cells of the cultured cell line it is not easy to relate any of these differences in growth rate, differentiated epigenetic or genetic properties specifically to the control of 5S RNA synthesis. The simplest explanation for the both the qualitative and quantitative (Table 2) correlation between translocation of a single gene cluster and loss of complete repression is that the two events are causally related. If this is the case we can conclude that oocyte-type 5S genes are normally repressed in somatic cells not because there is any lack of polymerases or factors required for their transcription or because of the presence of specific diffusible inhibitors of transcription but because they are located in regions of the genome, such as heterochromatic regions, not normally available for transcription in somatic cells. By translocation to a region, such as the nucleolus organiser, which is normally expressed in somatic cells these oocyte 5S genes are now available for transcription. Assuming this interpretation is correct, we conclude that control of oocyte 5S gene repression in somatic cells is not an inherent property of the 5S DNA itself but of the chromosome region in which the 5S DNA is located. It cannot be due to diffusible factors (inducers or repressors) which interact with the 5S genes themselves since, in the absence of a higher level of control, such factors would operate at all points in a nucleus, and position would not affect their expression.

Table 2 Average relative transcription rates per gene for 5S RNA in three different *X. laevis* cell types

Total number of 5S genes	24,000	Total number of gene clusters	18
Average number of genes per cluster	1,340		
Number of somatic genes*	1,340		
Number of oocyte-type genes*	22,660		

Cell type	Average relative transcription rate per gene† Somatic gene	Oocyte-type gene
Oocyte	1 (10%)	0 53 (90%)
Liver cell	1 (99%)	0.0007 (1%)
Tissue culture cell *a*‡	1 (85%)	0.01 (15%)
b	1 (85%)	0.35 (15%)

* Number of somatic genes per haploid genome calculated assuming somatic genes are at a single cluster of average size, the rest of the genes are assumed to represent oocyte genes.

† The average relative transcription rate per gene is calculated by dividing the proportion of each type of 5S RNA by the appropriate number of genes and putting the somatic rate equal to 1. We assume no difference is stability of the two types of 5S RNA.

‡ Average relative rate of transcription in tissue culture cells assumes either (*a*) all oocyte genes are potentially active or (*b*) only one oocyte gene cluster (of average size) is active.

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Proton magnetic resonance study of the hydration of glucose

THE solvation of sugars by water has not been extensively studied in spite of its biochemical importance. Relaxation rates for ^{17}O in monosaccharides have been used to study solvation[1,2], the revised[2] estimate of the solvation number being 5±1 water molecules for D-glucose and 2.5±1 for D-ribose at 5 °C, in agreement with 6±1 and 2.5±1 (respectively) derived from dielectric relaxation amplitudes. Again, compressibility data for aqueous sugars have been used[3] to derive solvation numbers for monosaccharides, with results ranging from 2.5 to 4.0 at 25 °C. One limiting factor in nuclear magnetic resonance (NMR) studies of aqueous sugars is that the rate of proton exchange is fast and no separate resonances from the hydroxyl protons appear. We now report that at low temperatures the exchange rate becomes sufficiently slow in the *p*H range 4 to 8 to enable us to detect resolved resonances from the individual hydroxyl protons. The results are interpreted in terms of strong hydrogen bonding between water and glucose, the effective solvation number being at least 10.

At room temperature the NMR spectra of aqueous sugars have only a single peak for water and sugar–OH protons, because of rapid proton exchange. On cooling to about 0 °C, however, separate peaks for all the sugar–OH protons are resolved (Fig. 1). Typical data for D-glucose are included in Table 1. (See Fig 2*a* for the structure and labelling for *β*-D-glucose.) The following significant facts emerge: proton exchange with water is relatively slow for all hydroxyl protons. All ROH protons resonate down-field of the water protons, the anomeric hydroxyl proton resonance being considerably down-field of the remainder. All resonances shift up-field on heating, the shifts being comparable with that for water protons. They also broaden, the anomeric hydroxyl protons being most temperature sensitive, especially that for *α*-D-glucose. The effect of added basic cosolvents is, in general, similar to that on the water protons, comprising a relatively rapid up-field shift (Fig. 3).

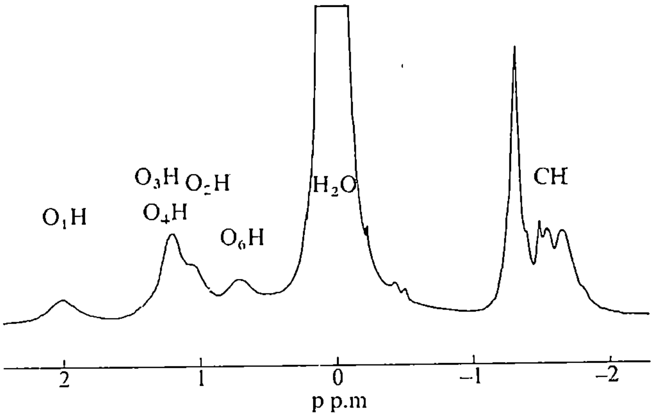

Fig. 1 100-MHz proton magnetic resonance spectrum of 2-molal α-D-glucose in water at − 6 °C.

Consider the solvated unit (Fig. 2*b*), in which two water molecules (also hydrogen bonded to other water molecules) are bonded to an OH group of a sugar molecule. Absence of (H_2O)α would give a $(O\text{–}H)_{\text{free}}$ group which would resonate at very high field values, so (H_2O)α must be present. Furthermore, (H_2O)*β* has a marked strengthening effect on the hydrogen bond to (H_2O)α, and its absence would shift the O–H resonance to a value up-field of the pure water value.

The results can be compared with those for aqueous alcohols. In these systems, separate hydroxyl proton resonances are not usually resolved in the water-rich region, but extrapolation of the shifts observed in the alcohol-rich regions leads to the conclusion that hydrogen bonding

Fig. 2 *a*, β-D-glucose; *b*, solvated unit; *c*, concerted proton migration; *d*, example of other structures likely to form as a result of continued proton transfer.

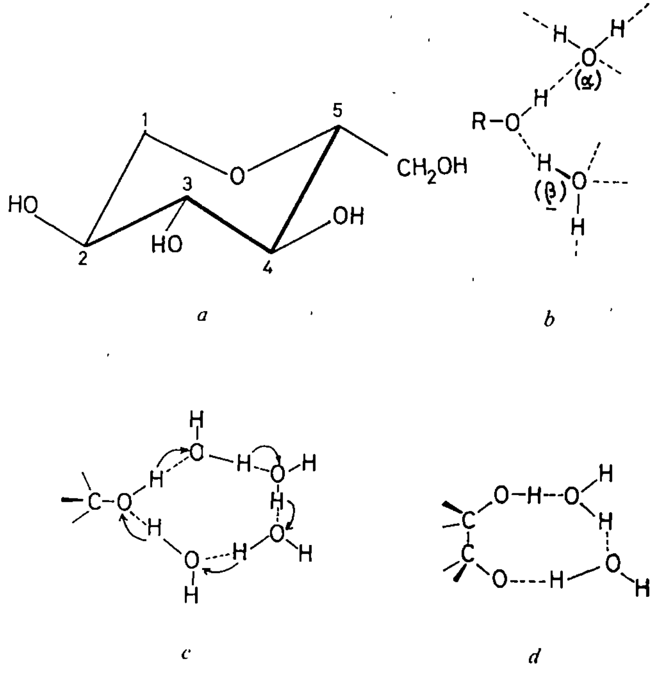

Table 1 Hydroxyl proton chemical shifts and temperature coefficients at 273 K

α-D-glucose (1 molal) Proton	Shift* (p.p.m.)	Temperature coefficient (p.p.m. °C^{-1})	β-D-glucose (1 molal) Proton	Shift* (p.p.m.)	Temperature coefficient (p.p.m. °C^{-1})
O_6H	−0.73	0.016	O_6H	−0.96	0.011
O_2H	−1.05	0.021	$O_{2,3,4}H$	−1.27	0.018
$O_{3,4}H$	−1.23	0.016		−1.43	0.018
O_1H	−2.05	0.011	O_1H	−2.87	0.013
H_2O§	0	0.010	H_2O	0	0.011

*Shift is down field from the water resonance at 0 °C.
†Temperature coefficient of MeOH is 0.009 p.p.m. °C^{-1}.
‡Assignment based on that for DMSO solutions[5] and the shifts obtained from aqueous DMSO (see Fig. 2*a* for numbering).
§Temperature coefficient of pure water is 0.0103 p.p.m. °C^{-1}.

between water and, for example, methanol, is actually more efficient than that between methanol molecules in the pure liquid[4]. The extrapolated resonance values are similar to those now reported for sugar hydroxyl protons. Thus, since it is generally agreed that methanol molecules in the pure liquid are nearly all doubly hydrogen bonded, this must also be true of methanol molecules in water and thus also of glucose in water.

The large down-field values for the anomeric hydroxyl protons are in accord with their relatively high acidities, as are their greater linewidths on heating. One of the most striking features of our results is the fact that proton exchange with water is relatively slow compared with simple alcohols and also with glycol and glycerol. We have found that other cyclic monosaccharides also give resolved hydroxyl proton resonances below 0 °C in aqueous solution, and thus suggest that the controlling factor is the cyclic conformation. This is supported by the fact that the exocyclic hydroxyl proton exchanges more rapidly than any of the others. We tentatively propose the following argument to explain this observation.

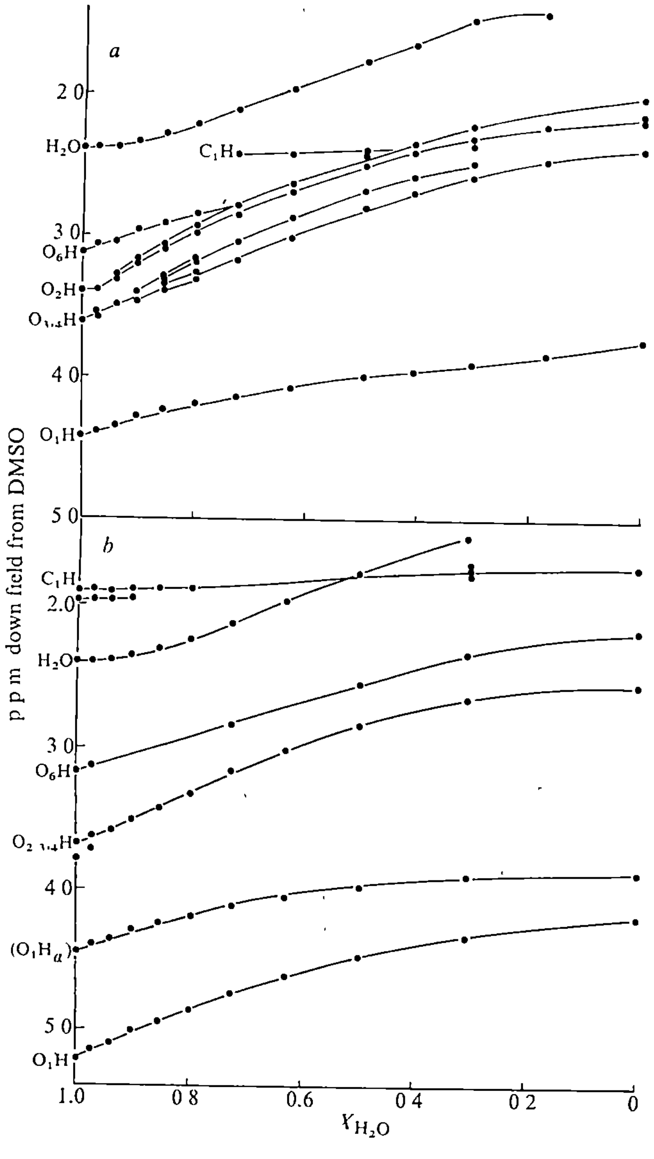

Fig. 3 Proton magnetic resonance of hydroxyl protons of α-D-glucose (*a*) and β-D-glucose (*b*) in water–dimethylsulphoxide mixtures at −6 °C. Hydroxyl proton shift against mole fraction of water. All shifts measured down field from methyl proton resonance of dimethylsulphoxide. Values were obtained using 1-molal glucose solutions (2-molal for pure dimethylsulphoxide).

Proton transfers of this type are acid–base catalysed, but in neutral solutions another, less efficient, process is probably involved, in which there is no charge separation. This can only come about by a form of concerted proton migration as depicted in Fig. 2*c*. Such structures are, of course, perfectly reasonable as fortuitous events in aqueous solution but, once formed, proton switching (as indicated by the arrows) will be facile. The lower the probability of such structures, the lower the rate of uncatalysed proton transfer. For $(CH_3)_3COH$, which does give a resolved O–H resonance at low temperature in water (our unpublished results), such structures are inhibited for steric reasons. This will also be true to some extent for the sugar hydroxyl groups, but another significant factor may be the tendency to form structures such as that in Fig. 2*d*, which cannot facilitate exchange, and which will compete with *c* and thus reduce the probability of *d* being formed. We suggest that these factors taken together explain the relatively slow proton exchange observed for sugar molecules.

The effect on the hydroxyl proton resonance of increasing the sugar concentration closely resembles that of adding methanol to the system, and the shifts are small. Dimethylsulphoxide and methylcyanide give rise to marked high-field shifts, because they remove the two hydrogen-bonded water molecules in turn, and only take the place of the one bonded to the hydroxyl proton (Fig. 2). Also, especially for methylcyanide, the resulting hydrogen bonds (ROH—$NCCH_3$) are relatively weak.

These results establish that, as anticipated, each hydroxyl group generally forms at least two hydrogen bonds to water.

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matters arising

Experimental analysis of seismonastic movement in the sensitive plant

I HAVE studied the irritable reaction to stimuli of the pulvinated leaves of the "sensitive plant", *Mimosa pudica* L. using filming techniques similar to those described by Charnley *et al.*[1]. Plants were grown in conditions very similar to theirs. Lighting was controlled strictly to a sequence of 14 h light (5,600 erg cm^{-2} s^{-1}) followed by 10 h darkness and the temperature was kept in the range 26.5±0.5 °C. I report here an analysis of the factors influencing the recovery of the primary pulvinus.

The behaviour of the leaf after a shock can be divided into two phases. First, the collapse lasts a few seconds, during which the pinnules fold together in pairs and the primary pulvinus sinks. Second, the recovery lasts about 1 h during which many workers have observed oscillatory behaviour[1–5]. A typical case is shown in Fig. 1.

For recovery, the curves of the variations of α against time can differ so much from leaf to leaf that many factors must influence turgor in the parenchymatous cells of the pulvinus. At least three factors are known to induce change in turgor in these cells—diurnal periodicity, phototropism and geotropism.

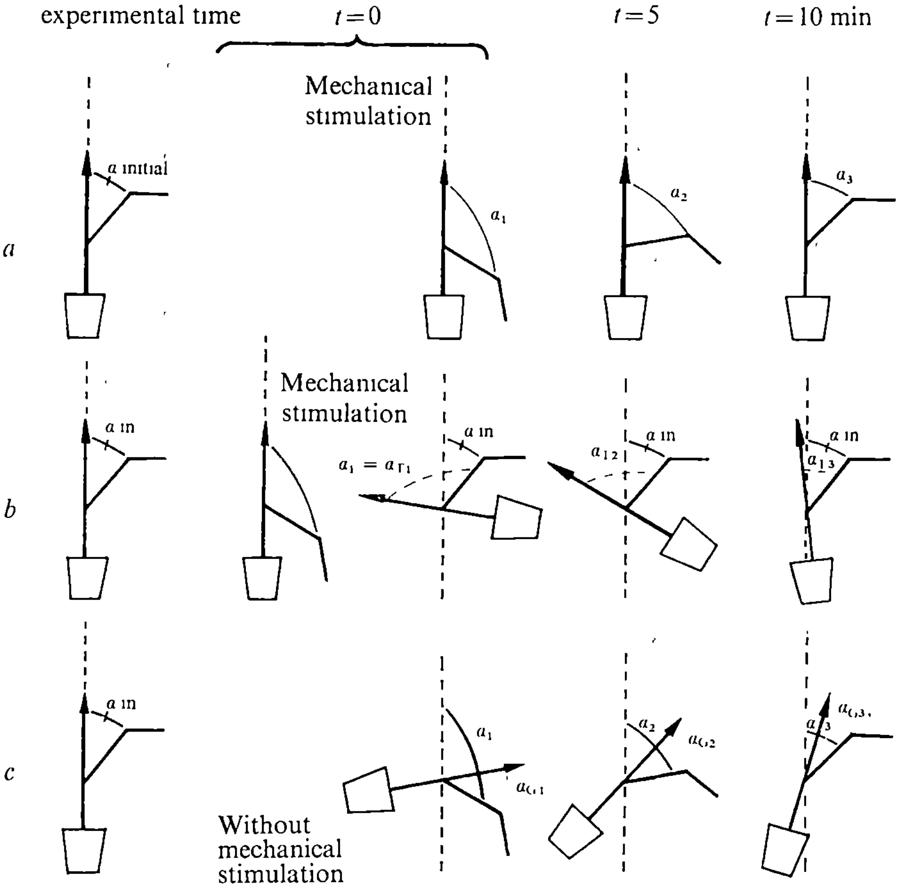

Fig. 2 Schematic representation of procedures used to determine the components of the recovery process of the primary pulvinus. *a*, Direct observation of recovery after mechanical stimulation quantified by angles α_1 and so on. This corresponds to Fig. 3*a*. *b*, Shock component measured by angles α_{T1} and so on when the geotropic effect is suppressed This corresponds to Fig. 3*b*. *c*, Geotropic component alone, measured by angles α_{G1} and so on. This corresponds to Fig. 3*c*.

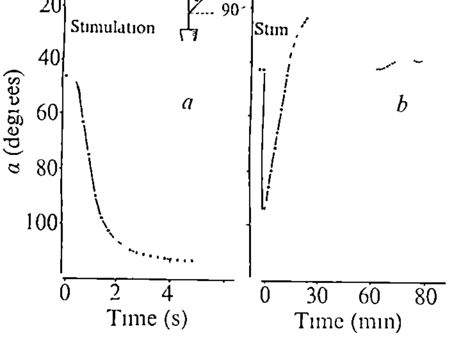

Fig. 1 Example of the time course in both phases of the seismonastic movement displayed by the primary pulvinus of the sensitive plant. *a*, Collapse; *b*, recovery.

The first factor manifests itself in variations with time of day of seismonastic sensitivity[6] and unstimulated angular position[5,7–9]. These variations are small during the time around the middle of the period of illumination. Diurnal periodicity can thus effectively be eliminated if all measurements are made during this part of the photoperiodic cycle, as was done during the investigation reported here. Phototropism was rendered negligible as diffuse fluorescent lighting was used. Geotropism, the tendency of part of a plant to orientate itself in a preferred direction with respect to gravity, is particularly marked in the sensitive plant[10]. If a leaf is displaced by carefully inverting the whole plant, it recovers its preferred orientation in 2 or 3 h But when a leaf sinks due to mechanical stimulation, geotropism will still operate as the petiole has lost its preferred orientation. At the same time shock is a major factor.

To measure the recovery component due to shock alone, a leaf was stimulated mechanically and geotropism was suppressed by maintaining the petiole at its initial orientation to the vertical throughout recovery by rotating the plant pot (Fig. 2*b*). The recovery component due to geotropism alone was determined as follows. First, I did a preliminary experiment with mechanical stimulation and with the plant pot fixed (Fig. 2*a*). Second, without mechanical stimulation, the pot was rotated each minute, to keep the angle between primary petiole and vertical the same as it was at the corresponding time in the preliminary experiment (Fig. 2*c*). These two procedures resulted respectively in curves (*b*) and (*c*) of Fig. 3. These two curves when added gave curve (*d*) which must be compared with the normal recovery (curve *a*). The agreement is adequate and, in particular, the corresponding maxima and minima do not differ by more than 2 or 3 min during the first hour of observations. This analysis explains the three apparently different modes of recovery reported by Charnley *et al.*[1] in different leaves of this species.

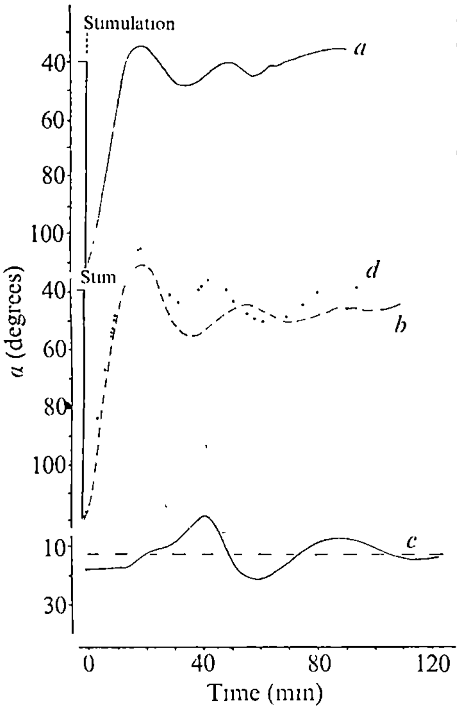

Fig. 3 Components intervening in the recovery process. *a*, Experimental temporal variation. *b*, Temporal variation due to seismonastic movement without geotropic effect. *c*, Temporal variation due to geotropism alone. *d*, Theoretical temporal variation obtained by adding curves (*b*) and (*c*).

These modes can be obtained by a simple relative shifting of curves (*b*) and (*c*), which has been proved experimentally.

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Crystal structure and Dirac's Large Numbers Hypothesis

TOWE[1] has raised what he considers to be a serious difficulty against Dirac's[2] Large Numbers Hypothesis. He claims that geological evidence weighs heavily against multiplicative creation. We point out here two serious flaws in Towe's arguments which invalidate his conclusion.

Towe claims that the measured equality of lattice dimensions between a 3×10^9-yr-old quartz crystal and a new quartz crystal weighs heavily against Dirac's multiplicative creation. Towe states that atomic distances in Dirac's theory vary like t^{-1}, t being cosmological time. Although it is true that atomic distances expressed in Einstein units do vary as t^{-1}, it is a conceptual error to use such a time dependence for comparison with measurement, since any observed value for distance is obtained with apparatus which measures only in atomic units.

In fact, Dirac's theory predicts that atomic distance scales formed from m (particle mass), e (particle charge), and h (Planck's constant) should not vary with t in atomic units, which are the ones to be used. Only the number of particles N, the gravitational constant G, and any quantity defined in terms of N and G (such as the radius of a star, or the orbit of a planet about a star) could depend on t when expressed in atomic units.

Even supposing that the lattice dimensions could vary with time, the only possible verification of this would be to perform the same measurement at two different times. The reason why measurements performed on two crystals at the same time must give identical results is simple. Both crystals are made of the same kinds of atoms with the same physical laws in effect—those laws which are valid at the time of measurement. Consequently, at any given time the lattice spacing for a given crystal structure must be the same regardless of whether the crystal is old or new; the past history of the crystal is irrelevant.

In point of fact, since lattice dimensions are expressed only in terms of m, e and h, which have no time dependence when expressed in atomic units (that is measurable units) Dirac's theory predicts that lattice dimensions do not change in time.

As mentioned repeatedly by Dirac[3,4], it is difficult to understand how the matter in very old rocks can have multiplied without disrupting their crystal structure. This is because N varies as t^2 ($M \simeq t^2$, because $M = Nm$).

Towe's experimental result that both the molecular weight and X-ray density of unit cells of the two crystals are equal tends to support the opinion that the new matter is not interstitial. This in no way constitutes a serious blow against Dirac's theory.

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TOWE REPLIES—Canuto, Adams, and Tsiang[1] are absolutely correct in pointing out that my crystal lattice spacing argument[2] regarding Dirac's multiplicative creation model is invalid. The lattice spacings of old and new crystals will indeed always appear the same. Nevertheless I cannot agree that no setback to Dirac's multiplicative creation hypothesis (in its present form) is incurred by the observation that there is a reasonable agreement between X-ray and pycnometrically determined densities in the minerals of the oldest rocks. If new atoms cannot be added as interstitials then they must be added to new unit cells external to and part of the existing crystal structures. About 40% additional atoms are required for crystals 3×10^9 yr old. In accommodating the added unit cells into existing rocks, any extensive deformation with accompanying phase changes (recrystallisation) must be avoided to prevent resetting the radioactive 'clocks'. The alternative to interstitials would thus seem to transcend conventional physics because in addition to the multiplicative creation of atoms with time ($Nm \simeq t^2$) it requires the simultaneous creation of the intercrystalline space ($V \simeq t^2$) to accommodate them. If this is accepted as a realistic alternative, then a rock and all of its crystals would be getting larger, resulting in a secular trend toward increasing grain size in older rocks. What inconclusive data there are[3] tend to support the opposite conclusion. Like Canuto's cosmological studies (in preparation), perhaps future petrographic studies can be designed specifically to deal with such an esoteric model of petrogenesis.

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Washington, DC 20560

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reviews

Developmental biology

Peter Calow

Crystals, Fabrics and Fields: Metaphors of Organicism in Twentieth-Century Biology. By Donna Jean Haraway. Pp. 231. (Yale University: New Haven and London, March 1976.) £9.

As with the organismic system she describes, Miss Haraway's book is a tight-knit fabric of several interdependent parts. There are three main threads. The first is a lucid, well-researched account of the intellectual development of Ross G. Harrison, Joseph Needham and Paul Weiss. The second uses the work of these men to re-examine the time-honoured controversy between reductionism and holism. Finally these two themes are brought together in a critical appraisal of the Kuhnian idea of scientific progress. The strongest thread in Haraway's argument is the first, the weakest is the third, and for me the most interesting is the second.

It has become fashionable to dismiss the part–whole controversy as 'stone-cold dead', and yet, Miss Haraway effectively brings it alive again by showing how it has played a crucial rôle in the work of three of the most important and influential developmental biologists of this century. In this context she asks. "Is the organicism–reductionism controversy, in which Harrison and Co. were involved, the same as the vitalism–mechanism controversy of yester-year": Miss Haraway wishes to establish a clear distinction between these 'four 'isms' because she wishes to argue that they represent the alternative paradigms to which developmental biologists were subjected in the 1920s and 1930s. I will argue that, in several crucial respects, she does not succeed in this task and that in consequence she fails to establish, beyond doubt, the legitimacy of the Kuhnian interpretation.

With Miss Haraway I take mechanism to be, literally, the machine view of life and vitalism to be the involvement of indispensable, non-physical entities in biological explanation. Reductionism rests on the belief that the whole can be fully explained in terms of the parts whereas organicism (biological holism) asserts that the whole cannot be fully explained in this way. Mechanism is equivalent to reductionism if, and only if, machines are reducible. Alternatively it is easy to see how through Drieschian arguments from self-repair and Paley–Polanyi-type arguments from design, that mechanism may lead to holism and vitalism —not, however, by a series of discontinuous, paradigm changes, as implied by Miss Haraway, but simply by following the metaphor to its logical conclusion. Similarly, organicism, although not being equivalent to reductionism, may be compatible with it. One must know about the whole before one can reduce it in any meaningful sense of the word. Surely, Harrison's holism was operational throughout; Needham began in this way but then progressed to a more uncompromising view of wholeness; Weiss seems always to have had anti-reductionist leanings. The discontinuities, if they exist at all, are most clear in the development of Needham. In spite of Haraway's lucid factual account, however, paradigm changes are not at all self-evident in the work of any of these men.

Haraway, as do the organismic biologists themselves, rejects the idea that organicism is equivalent to vitalism. And yet if one accepts an evolutionary cosmogeny in which whole is formed from parts, it is difficult to understand how properties could have emerged in the wholes which were not explicable in terms of the constituent parts. Otherwise as Vernon Pratt has argued in an important paper not cited by Miss Haraway (*Stud. Hist. Phil. Sci.*, **5**, no. 1, 1–13, 1974), it is necessary to postulate the coming into being of extra inexplicabilities between levels. But from whence do these inexplicables arise—like Aristotles *Pneuma*, from an extraterrestrial Quintessence? I suggest that there is in fact little difference between organicism in this extreme, non-operational form and the old vitalism, and that the former has evolved out of the latter.

Miss Haraway suggests that the strength of the Kuhnian approach is its concern for the extralogical, emotional aspects of science. This is also its weakness, for it is difficult, if not impossible to judge the paradigm idea in any dispassionate objective sense, and it is all to easy to make the facts fit the model. If revolution, discontinuity, crises, and so on, have been such important elements in the scientific advance, they should surely emerge as self-evident processes from the history of science. They do not emerge in this way from *Crystals, Fabrics and Fields.* In spite of this criticism, however, the book is still worth reading for its rich factual content, and were it not for the price it could provide a valuable adjunct to undergraduate, developmental biology courses. ☐

Dr Calow is a lecturer in the department of zoology at the University of Glasgow, UK.

Sedimentation within the tidal zone

Tidal Deposits: a Casebook of Recent Examples and Fossil Counterparts. Edited by Robert N. Ginsburg. Pp. xiii+428. (Springer: Berlin and New York, 1975.) DM 85; $34.80.

THIS volume, dedicated to the memory of Rudolf Richter for his pioneer research on tidal deposits, is an outstanding compilation of current knowledge of sedimentation within the tidal zone. Forty-five papers on selected recent and fossil tidal deposits are contributed by 52 authors, and it is refreshing to discover clear evidence of editorial authority in achieving a uniform style of presentation of the papers. Robert Ginsburg, in liaison with George de Vries Klein, John Harms and Richard Boersma, supplied authors with a definitive, itemised format, even adopting standard symbols for the preparation of facies diagrams. Thankfully, authors responded well in producing what are essentially a series of factual summaries of the principal facies types, vertical facies sequences, sedimentary structures, and faunas and floras characterising the various tidal deposits. All papers are copiously illustrated with photographs and invaluable graphical logs and facies maps. Consequently, this casebook provides sedimentologists with the necessary criteria for recognising and readily comparing sedimentary sequences deposited within tidal environments.

Two major subdivisions are considered—the siliciclastic and carbonate tidal deposits. Recent siliciclastic examples include the classic tidal flats of the North Sea, Mont Saint-Michel Bay and the Bay of Fundy, transgressive tidal deposits in barrier island complexes of the Atlantic coast of North America, fine-grained deposits on the margins of the Colorado River Delta, wind-tidal flats of Laguna Madre, and deposition within tidal inlets at Long Island and the Dutch coast. Twelve fossil analogues are described, ranging in age from Precambrian to Pliocene, but mostly from the Palaeozoic of North America. The examples vary from those showing many of the diagnostic sedimentary features of a North Sea model, to a type in which the sedimentary record shows little evidence of sea-level fluctuation.

Recent carbonate examples are naturally from the Bahamas, Arabian Gulf and Shark Bay, Western Australia, for which subtidal, intertidal and supratidal deposits are described. The 19 fossil counterparts are discussed in three sections—examples showing the three tidal zones of accumulation; those characterised by laminated and stromatolitic facies; and a miscellany illustrating how it is possible to recognise ancient tidal deposits on the basis of sedimentary features and facies pattern.

This unique casebook ends with an annotated bibliography of what are regarded as the 25 key papers on tidal sedimentation. **Brian Waugh**

Form from top to bottom

Orientation and Form. By Irvin Rock. Pp. 165. (Academic: New York.) £5.75.

THIS book is the outcome of twenty years research: it addresses two questions. First, what determines the orientation in which we see a visual form—that is, which part of it do we take to be the top? It could be the position of the form on the retina, its orientation with respect to gravity, or its orientation with respect to the visual framework. Usually all three factors coincide, but Rock has explored systematically what happens when we view forms with head tilted or inverted and when there is no visual framework. He has also shown that there are three further determinants of what we take to be the top of a shape—familiarity with the shape in a given orientation, the endogenous properties (such as symmetry) of the shape, and instructions. Rock gives a most convincing account of how these factors interrelate and of what determines which will predominate when they are in conflict.

The second question Rock considers is what determines whether a shape will be recognised when its orientation is changed, and here he successfully resolves two apparent contradictions in the literature. It is normally found that when the head is tilted, forms are seen the way up they are in space not the way up they are on the retina. Nevertheless, certain kinds of complex but familiar forms, like faces and letters, become very hard to recognise when inverted on the retina. He argues that it takes time to correct for changes in orientation on the retina caused by head tilt and that the correction process operates in a piece-meal fashion: it is therefore difficult to correct fully when a complex shape such as a face is presented in an unusual orientation. He cites experimental evidence of his own, much of which has not previously been published, to support his claim. He also gives a convincing explanation of why it is that some forms are most difficult to recognise when they are turned through 180°, others when they are turned through intermediate angles. He anticipates some of Shepard's more recent (and currently better known) work on the problem of orientation: in many ways Rock's insights go deeper and take into account a wider range of factors.

Orientation and Form is modest both in length and manner. It is an excellent example of how an approach based on common sense and carefully designed experiments can resolve important issues and lead to the construction of a convincing theory that is anything but obvious to common sense—except in retrospect.

N. S. Sutherland

Success in ion transport

Ion Transport in Plant Cells and Tissues. Edited by D. A. Baker and J. L. Hall. Pp. xiv+437. (North-Holland: Amsterdam and Oxford; American Elsevier: New York, 1975.) Dfl. 130; $54.25.

THE editors of this book have dealt successfully with a difficult problem confronting those who assemble a multi-author volume, the level and tone of address to the reader is relatively uniform. To have achieved this while preserving the individual styles of writing indicates how much unobtrusive work must have been done.

The level of address is fairly advanced, making the book most valuable to postgraduate students, research workers and teachers seeking an entry into the detailed literature. In several chapters there are summaries of the state of knowledge in the form of schemes and models, and there are useful compilations of information in tabular and chart form.

There is insufficient emphasis in many of the chapters on how experiments are carried out and not enough comment on the reliability of techniques. The experimentally-minded reader would have been helped more if the frailties of certain procedures had always been dealt with as straightforwardly as in excellent chapters by R. G. Wyn Jones (Ion Uptake in Excised Roots) and J. L. Hall (Cell Membranes).

In their introductory chapter Baker and Hall reproduce a most thought-provoking illustration, from a book compiled in 1959 by F. C. Steward, in which salt accumulation is related to overall processes of growth and metabolism. Nearly 20 years on it is disappointing how little attention several of the authors are prepared to give to this integrated approach. It is surely this reluctance, coupled with experimental convenience, that packs the book so full of information about the absorption of chloride, simply because it is *not* involved to any major extent with metabolism. It is regrettable that the coverage given to the published work on the absorption of the nitrogenous ions, and on phosphate and sulphate in relation to their metabolism is far from complete.

The book ends with three enjoyable chapters in which ion transport is related to specific functions (T. J. Flowers on Halophytes; U. Lüttge on Salt Glands; D. A. Thomas on Stomata). These, taken with two splendid chapters by J. B. Hanson and D. E. Koeppe on Mitochondria and P. S. Noble on Chloroplasts, suggest that it might have been better to have adopted this functional approach throughout the book rather than, in the middle chapters, to have described ion transport in higher plants according to the experimental approach by which it is studied.

The cost of this book, for all its merits, is a staggering $54.25. It must be a great disappointment to the editors that at such a price, the book will be beyond the financial reach of most individuals and many smaller libraries.

D. T. Clarkson

obituary

Raymond Bridgeman Cowles, Professor of Zoology at the University of California, Los Angeles, died on December 7, 1975, at the age of 79. Born in Natal, South Africa, he spent his childhood there but was educated in the United State, receiving his doctorate at Cornell University in 1928.

Two themes from his Natal boyhood were stamped on Cowles' subsequent career: first, the many animal-environmental relationships he saw in the bush near his home in the Umzumbe Valley, such as the monitor lizards that laid their eggs in the almost impregnable fortresses of termite nests, and second, the transformation of the Zulu to a state of deep poverty as their numbers increased. His career was further stimulated by the work he did to raise money for his education. he worked as a 'muleskinner' and as a canal guard in the then desert wilderness of the Imperial Valley of California.

His tools were the simplest of instruments: the camera, the thermocouple and thermometer, and his own critical powers of observation, yet he founded the field of thermal relationships of reptiles. He did more classic work on the pit-organ of rattlesnakes, where he demonstrated the exquisite thermal sensitivity of this receptor. Blindfolding the rattlesnake, he swung before it a glowing electric bulb encased in black cloth. Often enough, the snake struck at the "warm prey object", hitting it in midswing with a deadly-accurate spray of venom One had no trouble envisioning a leaping desert mouse or pocket gopher receiving the same treatment, all through central integration of afferent impulses from the most sensitive thermal receptor in the animal kingdom.

The same quality of insight led to the remarkable generalisation that male germ cells in diverse species are inactivated by exposure to approximately the same thermal levels. Central to this finding were observations that male reproductive tissues are protected from high temperatures in various ways; for example, the scrotum of mammals and the placement of gonads of birds adjacent to air sacs within the body. Cowles also saw that blood flow was responsive to temperature, and that this dynamic interaction was crucial to thermoregulation in reptiles, birds, and mammals, proving this by beautifully 'simple' experiments.

Professor Cowles also performed early work on water conservation and loss in desert animals and many other ecological subjects. Another dominant factor in his later career was his realisation that the human race violated the rules by which living things in nature subsisted in ecological harmony. A major book on this subject was just completed at the time of his death and awaits publication.

An important part of Cowles' writing was for the interested public, but he taught most of all by encouraging his students to participate in the excitement and insights of field trips, thinking with him as he pondered on what he saw. A motor trip through the desert might find him ranging across topics as diverse as the migration of waterfowl over established desert flyways, the economic use of the jojoba plant (which is now being used to provide a substitute for sperm whale oil), the dynamics of soil and sand movement in response to wind and water, the basking behaviour of rattlesnakes on warm pavements at dusk, or the aerodynamics of bird flight—the car's speed would be adjusted to that of a bird flying alongside, and its speed checked.

Professor Cowles was serenely unimpressed by his many academic accolades, and everywhere he was much respected for his deep honesty and warmth as a human being.

Kenneth S. Norris
Clara M. Szego

John Buchan Jepson, who died on May 7 aged 53, was the Professor of Biochemistry at the Middlesex Hospital Medical School.

After taking a first degree at Cambridge, he went to work for his doctorate at Oxford, where he did research on Penicillin under the supervision of Sir Robert Robinson. He later worked on the biochemistry of amino-acids, hydroxylases and psychomimetic amines, and also on hereditary diseases and chromatography. He was also very interested in Biochemical Education; he was always trying to find new methods of conveying the excitement, importance and relevance of his subject—and as enthusisasts always do, he succeeded in involving his audience. Another aspect of this interest was the efficient and rapid communication of research. He was chairman of the Committee on Biological Information, and worked right up till his death on new means of data dissemination.

announcements

Meetings

June 10, **Conservation of Wild Life in Kenya**, London (The Secretary, The Royal Society of Arts, John Adam Street, London WC2 6EZ).

July 5–8, **Mathematics of Hydrology and Water Resources**, University of Lancaster (The Secretary and Registrar, Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend on Sea, Essex SS1 2JY, UK).

September 6–9, **Nonlinear Waves and Solitons**, University of Newcastle upon Tyne (The Secretary and Registrar, Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend on Sea, Essex, SS1 2JY, UK).

September 14–16, **Theoretical Developments in Control**, University of Leicester (The Secretary and Registrar, Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend on Sea, Essex SS1 2JY, UK).

December 21–22, **The Structure and Assembly of Biological Systems**, London (Dr A. C. Allison, Clinical Research Centre, Watford Road, Harrow, Middlesex, UK).

April 18–22, 1977, **Fifth International Conference on Reactor Shielding**, Knoxville, Tennessee (Deadline for abstracts: August 20) (D. K. Trubey, Radiation Shielding Information Centre, Oak Ridge, National Laboratory, PO Box Y, Oak Ridge, Tennessee 37380).

July 11–15, 1977, **Veterinary Parasitology**, Sydney, Australia (The Secretary, 8th International Conference on WAAVP, CSIRO, McMaster Laboratory, PMB No 1, Post Office, Glebe N S.W 2037, Australia).

August 15–18, 1977, **Chemical Thermodynamics**, Ronneby, Sweden (Fifth International Conference on Chemical Thermodynamics, Chemical Centre, Lund University, PO Box 740, S-220 07 Lund, Sweden).

September 26–28, 1977, **On-Line Surveillance and Monitoring of Process Plant**, London (Deadline for abstracts: October 31) (Conference Secretariat, On-Line Surveillance and Monitoring of Process Plant, Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS, UK).

Person to Person

A self-contained one-bedroom apartment in a modern building, conveniently located for central and West-End London by public transport; central heating, carpeted throughout. Exchange for apartment or house in Toronto–Hamilton area. Dr L. Knight, 370 Dixon Road, Apartment 1004, Weston, Ontario M9RIT2 Canada.

Science and Music. Physics librarian compiling a comprehensive bibliography on the collaboration between science and music, from the 16th to 20th centuries, would like to exchange information with other persons interested in the subject. Information relating to unorthodox theories and contraptions is specially desired. Please write to James R. Parrott, c/o EMS Library, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

All relevant material on the biological and ecological effects of extremely low frequency radiation arc being sought by the NRC committee studying the US Navy's Project Seafarer

All material and references should be submitted in writing as soon as possible to Samuel Abramson, Committee on Biosphere Effects of Extremely Low Frequency Radiation, National Research Council, 2101 Constitution Avenue, N.W., Washington, D.C. 20418.

The Fleet Study Group, is interested in the formation and structure of the Chesil Beach; the sediment of the floor of the Fleet; the physico-chemical characteristics of the Fleet water and the biological communities of the Fleet Anyone who can provide information on these topics or who wishes to help the study, please contact the Secretary, Mrs J. M. Fitzpatrick, c/o College of Education, Cranford Avenue, Weymouth, Dorset DT4 7LQ.

There will be no charge for this service Send items (not more than 60 words) to Martin Goldman at the London office The section will include exchanges of accommodation, personal announcements and scientific queries We reserve the right to decline material submitted No commercial transactions

Reports and publications

Other countries

World Health Organization A Multi-Axial Classification of Child Psychiatric Disorders· an Evaluation of a Proposal By Michael Rutter, David Shaffer and Michael Shephers. P' 85. Sw.fr.18 WHO Food Additives Series, No 7. Specifications for the Identity and Purity of Some Food Colours, Flavour Enhancers, Thickening Agents, and Certain Food Additives Pp. v + 216 Alternative Approaches to Meeting Basic Health Needs in Developing Countries Edited by V Djukanovic and E P Mach (A Joint UNICEF/WHO Study) Pp 116. Sw fr 24 (Geneva WHO, London HMSO, 1975 and 1976) [242

World Health Organization International Agency for Research on Cancer. Annual Report, 1975 Pp 149 (Lyon International Agency for Research on Cancer, London HMSO, 1975.) Sw fr.12 [252

World Health Organization Public Health Papers, No 64. Health Economics—Report on a WHO International Seminar Pp. 44. (Geneva WHO, London HMSO, 1975) Sw fr 6. [262

United States Department of the Interior Geological Survey Professional Paper 823–A, B, C, D Paleozoic Corals of Alaska. By Michael Churkin, Jr, *et al.* Pp 75 + 28 plates (Washington, DC US Government Printing Office, 1975.) [262

The Dr. H. P. Heineken Prize. By C Bauer Pp 32. (Amsterdam. The Heineken Foundation, 1975.) [272

Environment Canada Fisheries and Marine Service Technical Report No 520 Two Computer Programs for Analysis of Catch-at-Age Data By W G Doubleday Pp 7 + POPI Listing (St Andrews, NB Research, and Development Directorate, Biological Station 1975.) [272

Food and Agriculture Organization of the United Nations. Cereal Seed Technology a Manual of Cereal Seed Production, Quality Control and Distribution. Edited by Walther P Feistritzer (FAO Agricultural Development Paper No 98) £2 60 Population, Food Supply and Agricultural Development. Pp v + 62 80p Report of the Fifth Session of the FAO Panel of Experts on Integrated Pest Control, held in Rome, Italy, 15–25 October 1974. Pp. 41 80p Land Reform Land Settlement and Co-operatives, No 1/2, 1974 Pp. 147 £1.60. (Rome FAO, London HMSO, 1975) [23

CERN—European Organization for Nuclear Research CERN 75–19· Instruction Timing for the CDC 7600 Computer By H Lipps Pp. 73 (Geneva CERN, 1975) [23

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Smithsonian Contributions to Zoology, No. 214 Myodocopid Ostracoda from Southern Africa By Louis S Kornicker. Pp 39 (Washington, DC: Smithsonian Institution Press, 1976 For sale by US Government Printing Office) [43

US Department of the Interior Geological Survey. Professional Paper 779 The Story of the Water Supply for The Comstock, Including the Towns of Virginia City, Gold Hill, and Silver City, Nevada, Together with Other Water-Related Events for the Period 1859–1969 By Hugh A Shamberger Pp xi + 53 (Washington, DC US Government Printing Office, 1972) 70 cents [43

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nature

June 10, 1976

Not a very good reshuffle

Dr Robert Press is one of the least self-assuming men you could ever expect to meet and so it is ironic that whilst his tenure of the post of Deputy Secretary (Science and Technology) in the Cabinet Office has been largely uncontroversial, his departure into retirement should be a matter of great interest and lead to wide speculation and considerable controversy. Not, we hasten to add, because Dr Press is being forced to retire for any reason. It is simply that the government is taking the opportunity of Dr Press's going to phase out any vestiges of a chief science advisory post in Whitehall. It is a peculiar quirk that this is happening just at the time that in the United States President Ford is reacquiring a science adviser after three years in which there has been no science policy office in the White House.

The announcement of the change comes in a memorandum which Lord Shepherd, the Lord Privy Seal, has just submitted to the Science Sub-Committee of the Commons Select Committee on Science and Technology. Some months ago the sub-committee discovered with some amusement that it was the Lord Privy Seal's responsibility to worry about inter-departmental cooperation and coordination on matters of science, and asked Lord Shepherd to submit a memorandum on the subject.

Much has happened in the past few years, of course, to readjust the balance of power in governmental science. Seven departments now have chief scientists to oversee a research and development budget totalling many hundred million pounds, and it was perhaps inevitable that as the science policy machinery grew in the departments the clout of the man in the centre, lacking research facilities of his own, should diminish. Now, says the Lord Privy Seal, the Cabinet Office's coordinating functions can be better fulfilled by an inter-departmental committee of chief scientists and permanent secretaries, whilst any scientific initiatives or advice to the cabinet can come from a scientist to be located within the Central Policy Review Staff (the so-called "Think Tank"). All these administrative changes may sound nit-picking and trifling, but to see what they mean in reality it is only necessary to remark that from now on the Prime Minister and Cabinet will be advised on science matters by the Head of the CPRS (Sir Kenneth Berrill) to whom a chief scientist will report. Since scientists are as status and salary conscious as everyone else, there will be some top rank people who will not consider the job as worth their while considering.

So the first problem will be recruitment. Is it to be the director of some government laboratory, asked to give up his rural environment for the suburban sprawl and a few hundred pounds extra per year? Or is some university professor, with moderate experience in policy-making, to be invited to surrender his chair and his research? In the United States people would be tumbling over each other for such a job, firm in the knowledge that it would open up new vistas; in immobile Britain there could well be difficulties in finding the right person.

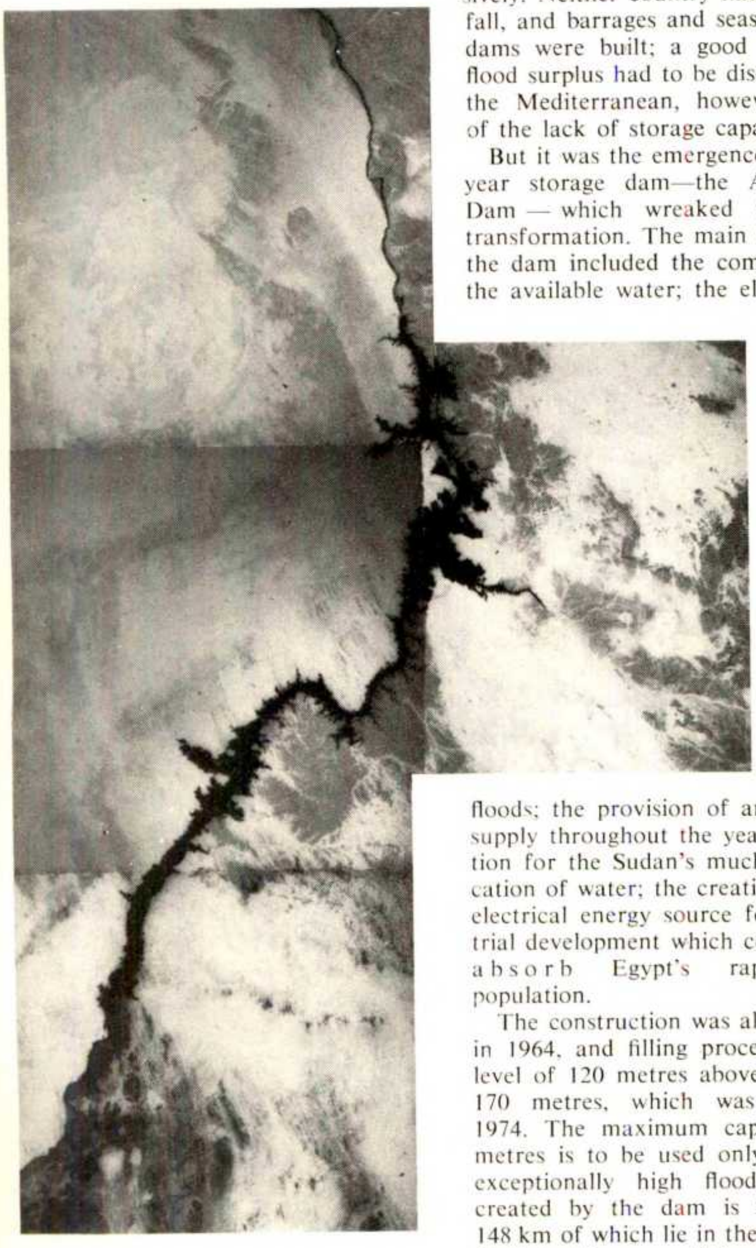
The second problem is that of access for the academic to government decision-making on science matters. Of course there are tens of thousands of scientists employed directly or indirectly by government, but science is a funny, complicated thing whose truths sometimes emerge in the most illogical ways. For government to take advantage of this it needs tentacles in all directions and particularly into the academic community. The worry is that in recent years, with the growth in the number of departmental chief scientists, too much scientific advice may be coming from within and not enough from without. The latest moves to put science into the CPRS do not bode well for a reversal of this trend.

The Lord Privy Seal also announces the formation of an Advisory Council for Applied Research and Development (ACARD). The justification for this committee of twelve or so, in which Sir James Menter will play a key role, seems to be largely to provide symmetry with the Advisory Board for the Research Councils which make recommendations on research policy in the pure sciences. ACARD will have no management functions and will simply provide advice to ministers on applied research and development in the public and private sectors. Its members, it seems, will only give it their part-time attention. There are already plenty of committees and structures devoted to applied research and development, not least the chief scientists, and it is difficult to see where ACARD has any significant new roles to play except to provide yet more committee work for the same people. Its appearance can hardly be greeted with much enthusiasm. □

A controversy reviewed

The arguments over the Aswan High Dam on the River Nile continue. **Julian Rzóska** offers this assessment

THE controversies which rage about large projects may not be resolvable in the final analysis, but at least in the case of the Aswan High Dam in Egypt they have been far from sterile. Hardly anyone seriously disputes the advantages which the taming of a once-erratic river offers in the form of regular and numerous crops and a constant supply of electrical energy. Equally, for many, some of the disadvantages have been painfully apparent—among them the waterlogging and salination of agricultural areas now deprived of fertile silt; the salination threat to fresh water areas in a Nile delta being eroded through a similar lack of silt; and reductions in off-shore fish populations.



The Aswan High Dam basin from space: the picture, taken in 1972, encompasses about 600 km of the Nile and about 75 % of the drowned area. The basin is now broader.

The Nile has been under some form of management since Ancient Egyptian times, of course, but it was in the nineteenth and twentieth centuries, when water needs rose sharply with the emergence of the Sudan as a consumer, that the river came to be used intensively. Neither country had much rainfall, and barrages and seasonal storage dams were built; a good deal of the flood surplus had to be discharged into the Mediterranean, however, because of the lack of storage capacity.

But it was the emergence of a multi-year storage dam—the Aswan High Dam — which wreaked the greatest transformation. The main objectives of the dam included the complete use of the available water; the elimination of floods; the provision of an even water supply throughout the year; compensation for the Sudan's much larger allocation of water; the creation of a large electrical energy source for the industrial development which could begin to absorb Egypt's rapidly rising population.

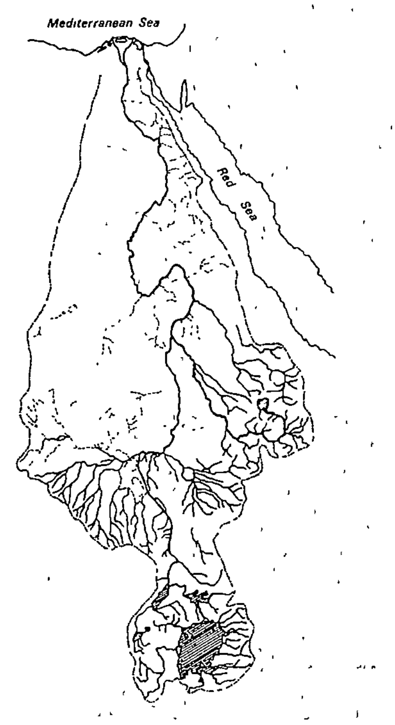
The construction was almost finished in 1964, and filling proceeded from a level of 120 metres above sea level to 170 metres, which was reached in 1974. The maximum capacity of 183 metres is to be used only at times of exceptionally high floods. The lake created by the dam is 500 km long, 148 km of which lie in the Sudan (Lake Nubia). The area of the lake depends on how full it is, but at 180 metres above sea level it would be about 6,000 km^2, with a volume of 156,000 million cubic metres. The lake's shape—oblong and relatively narrow with a dendritic outline—is typical of drowned river valleys; the shore, 9,000 km long, is bare desert.

It is not such statistics which have attracted attention, however. Indeed, few dam projects have drawn as much criticism as the Aswan High Dam, even before it was built, when the drowning of treasured antiquities was mooted. But with the dam basin now 10 years old, the Egyptians themselves have been taking stock of the main problems created by the dam, most significantly at a symposium in March at the National Research Centre in Cairo which dealt mainly with the effects downstream of the dam.

One of the main criticisms, for example, was that the dam was wasting water it was meant to save. A balance sheet, based on available hydrological documentation, shows the present situation, and is summarised in the table, presented by the chief consultant engineer Lennart Berg of Stockholm. Three important facts emerge: water available for the three areas of irrigated agriculture in Egypt and the Sudan amounts to 79,000 million cubic metres with the dam, as against 60,000 million without it; the big loss by evaporation from the lake is more than offset by the abolition of discharge into the sea; and the dam's buffering capacity allows for storage of the wide fluctuations (60,000 to 110,000 million cubic metres) of the Blue Nile floods.

The fate of the famous Nile sediments of the Blue Nile flood, which have built up the alluvial soils of Egypt, has provided the target for further objections. The chief limnologists of the project have thoroughly investigated this for the period up to 1973. Predictably, the sediments fall out where the current slackens and its "carrying capacity" is lost, and this happens in the southern part of the basin; 51 % of the fallout occurs between 410 km and 345 km from the dam, and the rest in the next 60 km, almost all of it in the Sudan. Silting up of the lake basin is a long way off, however. Cautious calculations give a figure of about 300 years for the sediments to approach the dam, although this figure is regarded by some as too high. As for the loss of the sediments' fertilising effect on the soils, the delta lakes and the eastern Mediterranean, the detrimental effects are already clear in the grave reduction of the sardine fisheries in the sea and a lesser yield of the delta lakes. The new lake has admittedly created inland fisheries, which in 1973 produced an output of 10,000-13,000 tons, but the long dis-

Illustrations from J. Rzóska (ed.), The Nile: Biology of an Ancient River, *by permission of Dr W. Junk Publishers, The Hague.*



Drainage basin of the Nile system. The dotted lines represent ancient water courses

tances from Aswan make this fish more expensive than that from the sea and the lakes. The soils themselves will need artificial fertilisers.

Erosion problems have undoubtedly increased with the water head at Aswan now standing some 50 m above the previous river level and in the absence of the protective layer of sediments. The threats of river bed erosion and to bridges and river banks, all of which have been forecast, are being closely watched, and so far no serious changes have been recorded. Threats to the coast line, which existed before the dam was built, of course, are now more serious, and a team of experts of the "UNESCO-Coastal Project 1973" based in Alexandria is monitoring developments. Another effect of the loss of sediments concerns the northern dam basin itself. Critics predicted that the basin would never fill because of seepage losses into the bare river bed. Careful measurements of the amounts of water needed to fill the basin confirmed a heavy loss, but this is gradually petering out. With a rich phytoplankton already developed, biological sediment will help seal the bottom.

The perrenial irrigation which now prevails raises problems of soil salination which call for more efficient drainage. These problems did not exist with basin irrigation, which was 'ecologically sound', but they are a common phenomenon in most countries relying on irrigation agriculture. The problems are most serious in the delta, which forms 60% of the cultivated land of Egypt. With a very small slope, artificial drainage by pumping will have to be increased. A change to different methods of irrigation—the sprinkler method used in Israel, for example—and the introduction of more salt-resistant crops have both been suggested, and are practised in many countries with similar irrigation problems

Other detrimental side effects that were forecast included the spread of water-borne diseases, especially schistosomiasis (bilharzia) and malaria. Bilharzia has been in Egypt for at least 4,000 years; the last serious outbreak of malaria in Egypt occurred in 1942. Because the shores of the new lake are still bare, they do not offer opportunities for snail or insect vectors. But the weed problem has to be watched, particularly the spread of *Eichhornia crassipes,* which is rampant in the White Nile south of the Gebel Aulia dam about 1,500 km south of the new lake, and also present in the delta. Eradication of *Eichhornia* is notoriously difficult and strict monitoring will be a necessity.

The main outcry against the High Dam, however, was that no gains could justify the cultural losses caused by the drowning of a large part of Nubia and its antiquities. The narrow riverain land of Nubia, which stretches from Aswan south to about the cataract IV in the Sudan, contained remains of a series of riverain civilisations from palaeolithic onwards. About one-third of the Nubian Nile valley is now drowned. Following the salvage appeal by UNESCO, intense activity by archaeologists rescued much in a short time; Abu Simbel is only one example, and overshadows equally important results—a large documentation on the prehistory of the region, for example, and the rescue of the remains of the flourishing Christian civilisation at Faras. But much is lost.

Furthermore, the strip of alluvial land surrounded on both sides by the desert was the home of 120,000 Nubians, differing in both origin and language from both Egyptians and Sudanese. They eked out a living through scanty cultivation along the river shores, and some fishing. Many had to seek employment in Egypt and the Sudan But they always returned to their homeland, and were intensely attached to it. This population, straddling the political boundary between Egypt and Sudan, had to be evacuated in 1964 before the advancing floods; those from the Egyptian part went to Kom Ombo, and those from the Sudan stretch were taken far inland to Khasm el Girba on the Atbara river. This caused much bitterness and serious social consequences.

But if the value of the Aswan High Dam is to be judged, an overall assessment of Egypt's situation is necessary. The population has increased from 10 million in 1900 to 36 million in 1975. Only about 3% of the total area of 1 million km^2 is cultivated, although a thorough search for more cultivable land has increased the existing 29,000 km^2 by one-third. Perennial irrigation has increased by a factor of 1.6 the cropped area (that is, yielding more than one harvest a year). With the increased population the water share per head has actually fallen, and birth control is difficult And the country lives almost exclusively on agriculture, because its industry is still very small. There are joint projects with the Sudan to search for new water resources—among them a canal bypassing the Upper Nile swamps with huge water losses, a very problematic project affecting the life of the Nilotic tribes of the Sudan. Other projects are under discussion.

The dam has brought Egypt a prospect for the redeployment of some of the population, as has the Roseires Dam in the Sudan. The energy potential of the Aswan High Dam is estimated at 8,000 million kW, which (other things being equal) will allow for some industrial development. The critics of the dam come from climatically and industrially far better-off countries, and must appreciate that Egyptians are aware of the dam's implications; their needs are great, and it is ultimately for them to decide how these should be met. For them, the controversies will presumably end at a time of their own choosing. ☐

Nile Waters during an average year
with and without the Aswan High Dam (in million m^3/year)

	With	Without
Water amount available in Sudan	85,000	85,000
Allocation to Sudan	−18,500	−13,000
Evaporation losses in new lake	−11,000	—
Water passing Aswan	55,500	72,000
Irrigation of Upper Egypt	−24,300	−18,800
Recirculation gains	+ 5,300	+ 8,000
Water passing Cairo	36,500	61,200
Irrigation of Delta	−36,500	−28,200
Discharged into Mediterranean	—	−33,000
	0	0

NUCLEAR TRADE: FRANCE

Seven days in May

At the end of last month, just days after Iran had confirmed that she is to buy two nuclear reactors from France, a French consortium unexpectedly won the lucrative contract to supply two nuclear reactors to South Africa. Chris Sherwell reports

THE independence for which France has striven in her nuclear policy since the days of de Gaulle used to be confined largely to the realms of defence and the nuclear deterrent—the *force de frappe*. But times change The last week in May was enough to confirm that, even as France assesses the balance of nuclear and conventional weapons in her defence strategy, an independent stand remains possible—in the equally sensitive field of nuclear trade, where the international consequences promise to be more widespread.

The timing of the Iranian and South African deals, as much as the content, reinforces the growing image of independence Apart from coming ahead of important secret meetings in London last week of the expanded "Group of Seven" nuclear exporting countries, they closely followed the Canadian decision to end nuclear cooperation with India (see following story), and were announced in the face of growing US suspicion of France's controversial plans to sell nuclear reactors to Libya. The news of the South African deal also came just days after President Giscard d'Estaing's US visit, and not long after he held summit talks with leaders from the countries of Francophone Africa who gathered in Paris earlier last month.

On both occasions, it is likely that the possibility of the French consortium of Framatome, Alsthom and Spie Batignolles actually winning the $1,000 million South African contract was mentioned, if not elaborately discussed. Anticipating criticism when the announcement finally came, the deal's defenders inside France have been quick to martial their defences They point to domestic employment and balance of payments benefits, but on a wider front face the problem that South Africa (like France itself but unlike either Iran or Libya) has not signed the Nuclear Non-Proliferation Treaty and has in fact recently reaffirmed her commitment to the nuclear option—facts which have enhanced the fear that South Africa might by the deal be acquiring a nuclear weapons capability

Far from stressing that France's nuclear trade policy does include safeguards, however, French officials maintain that no sensitive materials demanding safeguards are actually involved . in the words of one government spokesman, the contract is for the sale of "mere producers of electric current"; a spokesman for Framatome adds that plutonium produced by the two pressurised water reactors is not suitable for military purposes, and the point is also being made that South Africa has already developed the critical techniques of uranium enrichment and of fuel reprocessing anyway The affair, says the government, should be viewed as "strictly commercial and technological"

That political factors helped crucially to decide where the main reactor contract should go is, however, openly acknowledged. The talk is of France's "reliability"; France's pragmatic foreign policy, particularly in respect of South Africa, is highlighted as a factor making the deal possible. Certainly it appears that South Africa made a judgment, probably in light of France's past arms sales to her, that while the deal would demand justification, French public opinion was unlikely to prevent it going through.

Right or not, that judgment may have been vindicated by reports on the imminence of a South African decision which stated that the leading candidate was a US-Swiss-Dutch consortium. Attention was thus helpfully drawn to the USA, where the chairman of the Africa sub-committee of the Senate Foreign Relations Committee examining aspects of the deal thought it contradicted the USA's Southern Africa policy, and to Holland, where an anguished coalition government prevaricated over the problem of its share of the credit guarantees Certainly the proffered view of French officials is not in accord with the assessment of many observers that the French consortium won the contract by simple default when the Dutch government failed to meet its deadline

For all that, the French press, French protestant churches, parties of the left and groups like Friends of the Earth have reacted strongly to the deal since its announcement. Reaction outside France has also grown, particularly in Africa, where the Organisation of African Unity and the African National Congress in South Africa were each quick in their condemnation. Reaction in Holland was understandably more mixed, since the impression was about that the deal had been allowed to slip from the country's grasp—some thought by deliberate government contrivance The Dutch government successfully survived an opposition censure motion, however

The achievement will almost certainly contribute to the increasingly controversial debate now going on about international nuclear trade Iran's prime minister has already denied any intention of manufacturing nuclear weapons, saying Iran is interested in nuclear power "only in order to step up electricity production rapidly and avoid wasting oil". But he has also said that Iran's 1974 agreement with France covers the whole range of nuclear technology, implying that reprocessing facilities might eventually be wanted as well For her part France emphasises that any reprocessing facilities she provided would be controlled.

It is over the trade in both reprocessing and uranium enrichment facilities that the chief differences in nuclear export policy have arisen between France and West Germany on the one hand and the US on the other. The US would prefer a ban on such trade, while France and West Germany want trade but with safeguards and guarantees, including inspection, to prevent the misuse of the technology. As France's foreign minister puts it, for example, international life would become impossible "if one took it as a principle that international guarantees are worthless". But the Dutch reaction to the outcome of the South African deal is for many people more than enough to indicate that cut-throat competition would threaten the chances of safeguards achieving their objectives.

Already, though, the politics surrounding the latest deals has stretched far beyond the involved parties themselves and beyond questions about controls over a proliferating trade. Iran's prime minister has also had to dismiss suggestions, made in light of South Africa's existing links with Tehran and Mr Vorster's recent visit to Israel, of an emerging nuclear axis encompassing South Africa, Israel and Iran.

Similarly, there is the widespread view that the US is seriously considering what sort of support, both political and economic, it could offer South Africa in exchange for pressure from Mr Vorster on Rhodesia and movement over Namibia Part of this support, it has been suggested, could come in the form of various types of nuclear assistance; the fact that the State Department had sanctioned the contract which has now gone to France is cited in support of this. General Electric, leader of the tripartite consortium bidding for the contract, is moreover already in on the project. It may not be building the reactors, because the South Africans' own facilities will be on stream too late for the purposes of the project, it will be supplying them with the enriched uranium

- In spite of mounting safety doubts and spiralling costs France reaffirmed its commitment to nuclear power by confirming in April that it is going ahead with its £500 millions Superphenix fast breeder reactor programme. Defending the decision, which followed earlier protests from concerned government scientists and nuclear power trades unionists, the Industry Minister, Michel d'Ornano, stressed the official view that "nuclear reactors currently present a remarkable record in the matter of safety". The French Government is aiming for an installed nuclear capacity that will eventually meet almost all French generating demands, and if the 1,200 MW Superphenix prototype planned for the Rhone proves successful commercial breeder reactors could be under construction by 1990. Through the state-owned Electricité de France, France is a 51% partner in Superphenix with Italy and West Germany.

NUCLEAR TRADE: INDIA

An end or a beginning?

Canada recently decided to terminate nuclear cooperation with India. Our correspondent in Jullundur gives this assessment of the impact in the subcontinent.

ALTHOUGH Canada's decision was not entirely unexpected, it did come as something of a surprise because only in March this year, after nearly two years of strenuous negotiations between the two countries which had included three rounds of formal, two rounds of technical-level and several rounds of ministerial-level informal discussions, officials from the two sides initialled a detailed agreement in New Delhi which the two governments were expected to approve.

It was in this light that India responded. Speaking in the Lower House of Parliament, India's External Affairs Minister, Shri Y B Chavan, expressed disappointment and described the Canadian move as a "unilateral abrogation" of several provisions of the 1963 and later nuclear cooperation agreements between the two countries. He regretted that the Canadian Government had decided to "turn its back on the negotiated settlement and its contractual obligations" The Government of India, he said, was examining "the various implications" of the Canadian decision and would take "appropriate steps after this review has been completed". He made it clear, however, that there was "no ground for any suggestion that the Government of India is in any way responsible for ending Indo-Canadian nuclear cooperation"

Canada's Secretary of State for External Affairs, Mr Allan MacEachen, conveyed his government's decision to his Indian counterpart on May 18—exactly two years after India had carried out an underground nuclear explosion in the Rajasthan desert. This event is at the heart of the nuclear breach between the two otherwise very friendly countries as Mr MacEachen put it, it was evident that Canada and India had taken profoundly different views of what should comprise peaceful applications of nuclear energy by non-nuclear weapon states.

What effect will the Canadian decision have on India's atomic power programme? The worst that could happen would be more delays for projects that had already been delayed by an earlier Canadian ban on all nuclear shipments to India (of materials, equipment and so on) which was announced and enforced following India's explosion in 1974. The projects most affected by this ban were the second unit of the Rajasthan Atomic Power Project, RAPP-II (RAPP-I has been delivering power for quite some time now), the Madras Atomic Power Project (MAPP) and, to a lesser extent, the heavy water plant at Kota in Rajasthan, which is being built with knowhow developed at the Bhabha Atomic Research Centre (BARC) in Bombay.

For all of these projects India had placed orders with some Canadian manufacturers of special equipment, and sought alternative sources following the Canadian Government's ban. For example, critical components like reactor vessels and end-shields for MAPP reactors—originally on order from Canadian firms—have since been designed in India and are being built indigenously. The reactor vessel for MAPP-I has in fact already been delivered. Aside from a small number of specialised items which the country found it uneconomical to produce locally, most of the equipment and materials (including uranium fuel assemblies) for 200 to 250 MW CANDU reactors are being produced indigenously. RAPP-II, which after earlier delays will now be ready for commissioning in early 1977, may not possibly be able to go on steam as scheduled for want of enough heavy water. However, some heavy water is already being produced in the country at Nangal in Punjab (14 tons a year); a plant at Baroda in Gujarat (67 tons a year) was recently commissioned and is expected to start production shortly; and two more heavy water plants (Talcher, 62 tons a year, and Tuticorin, 71 tons a year) are expected to be commissioned by 1977. Thus, apart from the delays caused by the ban, any additional delays could only be marginal as far as projects already under way are concerned.

The feeling in India is that Canada may suffer a loss of credibility by its action, and eyes are now being turned towards the US, where the government is under some pressure to default on its contractual obligation to supply India with enriched uranium for her Tarapur Atomic Power Station (TAPS), built by an American company on a turnkey basis Shri Chavan has warned developing countries against attempts by developed countries to make participation in the technological revolution difficult: ". the Canadian thing", he told the Upper House, "is a warning in this direction", a warning to all developing countries and not only to India. India, he said, did not believe in making nuclear weapons, but it would not want to give up its right to develop nuclear energy for peaceful purposes.

The Canadian decision may yet help India move faster and with greater determination towards self-reliance and self-sufficiency in the nuclear field. Work has reportedly been going on at BARC on enrichment of uranium, for instance, and at TAPS on experiments aimed at finding ways to use plutonium in place of enriched uranium in the TAPS reactors How much progress is being made is not known, however. Canada's action meanwhile raises questions about safeguards. When the agreement was signed between India and Canada in 1963 for setting up RAPP-I, Canada wanted India to agree to inspection by the International Atomic Energy Agency (IAEA), but India did not agree. An understanding was reached whereby Canada could inspect India's RAPP reactors and India could in turn inspect Canada's Douglas Point reactors, but when Canada later supplied heavy water for commissioning RAPP-I, it insisted that India sign a trilateral agreement involving the IAEA which put RAPP-I under joint safeguards. India is now presumably free to refuse any inspection of RAPP-I by Canada or by the IAEA, and free to use plutonium generated in the RAPP reactors any way she chooses. India has, after all, been using her own uranium in her reactors, including RAPP-I, only TAPS uses enriched uranium supplied by the USA under a contract. ☐

Research budget setback for NSF

The Ford Administration's plans to boost spending on basic research in the United States have collided with election-year politics in the House of Representatives, and it now seems likely that support for many areas of research will continue to be eroded by inflation at least for another year. Colin Norman reports from Washington

THE House Appropriations Committee, the most powerful of the Congressional budget committees, has approved an appropriations bill for the National Science Foundation (NSF)—the chief source of federal support for non-biomedical basic research—which would reduce the foundation's proposed research budget by nearly $60 million. The bill would provide $681 million for NSF's research programmes next year, a 6% cost-of-living increase instead of the 20% boost proposed by the Ford Administration.

The bill will reach the floor of the House during the last week of June, but few upward revisions of the figures should be anticipated there. The Senate will then have its say on the measure, and although it has traditionally been more generous than the House towards NSF, it seems certain that the final, Congressionally approved, budget will fall far short of the Administration's request.

Why is Congress taking such a parsimonious attitude towards NSF? The answer is complicated but, essentially, the Appropriations Committee did not buy the Administration's arguments for increasing research support at a time of general fiscal restraint.

Dr. Guyford Stever, the director of NSF, stated the Administration's rationale most clearly when he described the NSF budget request to reporters last January. "The specific aim" of the proposed 20% boost in NSF's research support, he said, "is to

- In a report sharply critical of some of the policies of the Energy Research and Development Administration (ERDA), the Congressional Office of Technology Assessment (OTA) has concluded that "achieving energy independence by 1985 has become all but impossible". Moreover, since 37% of the oil consumed in the United States last year was imported and the proportion has now reached about 40%, OTA suggests that "even to hold import dependence to the present levels through 1958 would be a formidable achievement. The energy situation is serious and deteriorating".

The report, an analysis of ERDA's programmes and budget requests, suggests that although ERDA has made substantial progress during its first year in forging an energy research and development strategy, there are still serious deficiencies in its approach And there are even more serious defects in the budget allocations approved by the Office of Management and Budget (OMB).

In particular, OTA argues that ERDA is paying insufficient attention to policies which could affect the energy situation in the next five years. Although ERDA's recently announced energy plan places greater emphasis on conservation, OTA points out that such efforts still account for only 3.8% of ERDA's budget. Moreover, "the conservation programme has no overriding sense of direction", OTA charges. As for solar energy, OTA suggests that the budgets proposed for solar programmes—particularly the development of heating and cooling units—are much too small.

In many instances, the fault lies not with ERDA but with OMB. ERDA officials argued, for example, that conservation efforts should receive some $193 million in the fiscal year which begins on October 1, but OMB allowed the agency to request only $113 million in its budget application to Congress. Similarly, OMB sliced deeply into ERDA's proposals for solar energy before submitting the agency's budget to Congress.

OTA's analysis has already reached sympathetic ears in the Congress. Though the study was only published last week, drafts have been circulating around Capitol Hill for some time, and they have been used to brief members of key committees. Consequently, on May 25, the House Appropriations Committee approved a bill for ERDA which added $50 million to the request for solar energy development and $10 million to the budget for conservation efforts.

- In a decision hailed by various nuclear critics, a Federal Appeals Court in New York last week ruled that plutonium cannot be used as a reactor fuel in the United States until the federal government has completed a thorough study of safety and other issues surrounding its use. The immediate impact of the decision will be to delay the start-up of the only plant designed to reprocess commercial reactor wastes in the United States, but the chief effect could be to put off the recycling of plutonium for several years—a pause which nuclear critics are likely to put to good use.

Last November, the Nuclear Regulatory Commission (NRC), the agency responsible for regulating the nuclear industry, announced its intention to issue temporary licences to allow plutonium to be recovered from reactor wastes and recycled as a fuel, pending completion of a massive environmental impact statement on the matter. NRC's decision would have enabled a reprocessing plant, located at Barnwell, South Carolina, to begin limited operations next year. Though NRC made clear that the temporary licences would be revoked if the environmental impact assessment indicates that plutonium recycling is undesirable, nuclear critics were concerned that commercial plutonium use, once started, would be difficult to stop.

The Appeals Court agreed. It directed NRC to complete its environmental study—now scheduled for August—and to conduct public hearings on the issue before approving any licences. The whole process is unlikely to be completed until late next year.

Plutonium recycling is important to the nuclear industry (a fact underlined by the appearance in court of 28 nuclear and electric utility companies) because it will eventually supplement dwindling supplies of uranium. It will also be vitally important for a nuclear programme based on the breeder reactor. But there is widespread concern that commercial use of plutonium will greatly increase the chances of diversion of fissile material to clandestine nuclear weapons, and there is also concern about the health hazards associated with plutonium use. Moreover, it should be noted that the United States is trying to persuade nuclear exporting countries not to transfer reprocessing plants to countries wich do not already possess nuclear weapons, arguing that plutonium reprocessing at present is uneconomic. A decision by the United States to allow domestic reprocessing and plutonium recycling would be viewed abroad as hypocritical, at best.

counteract the gradual decrease of federal support for basic research, which has declined by about 23% in terms of constant dollars since 1968". Although research bugets have been going up steadily since the early 1960s, inflation has been increasing even more sharply and the purchasing power of the research dollar has consequently been shrinking.

Those trends were documented earlier this year in a study prepared for the National Science Board, NSF's policymaking council. The study, *Science Indicators 1974*, was said to have been particularly influential in persuading the White House Office of Management and Budget (OMB) to back the proposal for a large expansion of federal support for basic research. It showed that total expenditure on basic research (both public and private) increased from $1,200 million in 1960 to about $4,000 million in 1974, but that with inflation factored in, research support peaked in 1968 and by 1974 had fallen to the 1965 level.

In its written report on the NSF appropriations bill, however, the House Appropriations Committee didn't agree that basic research is in financial trouble, suggesting instead that "there may be problems of interpretation of the data" in the Science Indicators study. In particular, the committee notes that during the early 1960s, federal agencies tended to maximise their reported support of basic research but more recently, when the watchword has been "relevance", they have tended to classify research as applied whenever possible. Those factors have tended to skew the trends. Equally important, the committee argues that research budgets rose very sharply in the early 1960s, to reach a high level of federal support, and "this base has continued for the past decade". Moreover, the committee quotes Dr Stever as describing the United States' research and development effort as "still the strongest in the world".

In short, "after carefully considering all factors relating to NSF's research support programs, the Committee feels that the Foundation should be given the budgetary resources needed to continue its current level of research support". In addition, the committee notes that since the research budgets of a few other agencies, such as the Environmental Protection Agency and the Energy Research and Development Administration, have been increased, there seems to have been a marked shift in basic research priorities. The broad policy implications of that shift need to be studied by the new White House Office of Science and Technology policy and, perhaps, by the next Administration, the committee argues.

It should also be noted here, however, that for the past couple of years, NSF has been accorded considerable adverse publicity because of its support of supposedly trivial research projects. Several Senators and Congressmen have managed to get their names into the newspapers by taking cheap shots at research grants with funny-sounding titles and, consequently, there is a strong public perception that the foundation is wasting taxpayers' money. The committee notes in its report that there have been "major concerns in management and administration of NSF programmes", but it stops short of saying out loud that it isn't politically very easy to increase NSF's budget in an election year.

As for other parts of NSF's budget, the House Appropriations Committee was more generous. It increased, by $9 million, the Administration's budget request for science education activities, including a $3 million addition for courses to acquaint teachers with new school science curricula and new teaching methods. The bulk of NSF's education funds are channelled into universities and colleges, and provide a jealously guarded form of support for higher education. They therefore have considerable popular appeal, which is one reason why the committee preserved them from the axe.

Meanwhile, on May 27, the Senate passed a bill which would broaden NSF's activities and change some of the foundation's management practices. Sponsored by Senator Edward Kennedy, the bill would, among other things, increase public participation in the formulation of NSF policies by increasing the numbers of lay members on NSF committees and advisory panels. It would establish programmes to improve opportunities for members of minority groups to study science and engineering, and it would also establish a new programme of grants to enable state governments to strengthen their science policy machinery. A House-passed version of the bill is, however, much less ambitious and it is likely that some of the provisions in the Senate version will be dropped before the measure is given final Congressional approval. Some NSF officials have also expressed reservations about Kennedy's bill, arguing that it would steer the Foundation further away from its central mission of supporting basic research.

The chief worry of NSF officials, and their clients in the universities and colleges, however, is that Congress now seems likely to prolong the 10-year erosion of support for basic research.

AUSTRALIA

Back to two o'clock

Peter Pockley reports from Sydney on developments involving the institutions concerned with Australia's science policy

In spite of strong pressures for its abolition, the Australian Science and Technology Council (ASTEC) has survived. The Prime Minister, Mr Malcolm Fraser, has announced that the council, established last year by the Whitlam Labor government, will be reconstituted and continue for a further 12 months in an interim capacity. ASTEC, then, becomes one of the few of Labor's initiatives to survive the Fraser razor, and the scientist-politicians involved in the fight for ASTEC can be satisfied with their efforts. Survival was necessarily their prime goal, but it appears that this has been offset by the acceptance of uncertainties about the Council's functions and influence. The real bureaucratic battle has yet to be fought and it looks likely that ASTEC, in its reconstituted form, wil have no influence over the 1976–77 Budget priorities.

The science policy clock started ticking in Canberra about four years ago when the Labor Party became the first political party in the country to enunciate a science policy. Before then, the Liberal and Country Parties, through successive Ministers for Education and Science including Mr Fraser, had repeatedly set their faces against defining, or even attempting to define, a science policy. Spurred on by leading scientists to respond to the ALP initiative, the Liberal government of Mr William McMahon appointed an 11-man Advisory Council for Science and Technology (ACST) in the last months of the government's life. The clock advanced to one o'clock.

On Labor's accession to power in December 1972, the Department of Science was formed. The clock spurted ahead to four o'clock, but dropped back to three early in 1973 when the ACST was axed and no replacement body for advice was appointed. The clock stopped dead for a year while science policy, finance and administration marked time. The visit in 1974 by the Organisation for Economic Co-operation and Development (OECD) to study Australian science policy started the clock again, and by early 1975 it had reached six o'clock with the an-

Fight on his hands: Senator James Webster, Minister for Science

nouncement that ASTEC would be born. By the year's end the hour hand had started to climb up to base with ASTEC starting to sort out its priorities and gearing up to offer effective advice. Seven o'clock had been reached with the longest hours of labour ahead before a coherent set of principles and proposals could be formulated.

Then, in December, Labor fell; time went into reverse. Mr Fraser announced the retention of ASTEC before zero was reached; the time is now two o'clock, a small but definite advance on the Liberals' last position on the formulation of science policy. Here the temptation must be resisted in trying to stretch the analogy further, lest it turn into a clock paradox.

ASTEC's Secretariat has been transferred in body and soul from the Department of Science (where the mutual relations were markedly uneasy) to the Department of the Prime Minister and Cabinet; it has the status of a bureau and will report directly to the Prime Minister. The question of statutory independence has been postponed to the end of 1976, and although not generally favoured by the Liberal's approach to controlling advisory bodies, has not been ruled out. The Minister for Science, Senator James Webster, has publicly accepted the move although it has notably downgraded his role in Cabinet and his Department's chances of survival. For example, the Advisory Group of five men set up to examine ASTEC earlier this year accepted lock, stock and barrel the terms of reference spelled out by Mr Fraser himself during the December 1975 election campaign, *viz*:

> The Council will be the major independent advisor to Government on such matters as:
>
> 1. The development and application of science and technology to national needs and objectives.
>
> 2. New areas of science and technology which are of importance to Australia, including fields of industrially and commercially oriented research and development.
>
> 3. The balance, adequacy and effectiveness of national efforts in various fields of science and technology, including defence science, and means for improving efficiency in the use of resources.
>
> 4. The relative importance of efforts in those fields of science and technology which may contribute to national economic and social development and welfare and to the advancement of scientific knowledge.
>
> 5. The effective development and utilisation of scientific and technological manpower.

The first Interim ASTEC had established a number of Task Forces. These have been formally scrapped in name only, for "ad hoc committees and working parties will have to be used for specific tasks . . . Before establishing a committee or working party the Council should obtain Prime Ministerial approval". Maintaining a fine balancing act between independence and being firmly in Mr Fraser's pocket may be helped by a provision for "early publication of Council reports . . . for comments from the scientific and the industrial community before firm decisions on policy are taken by Government".

A further period of uncertainty and delay in bringing ASTEC to a full flowering has been ensured, though, by Mr Fraser's direction that "the first task of the Council will be the preparation, by the end of 1976, of a definitive report . . . on the long-term future of ASTEC." Self-examination, again.

Six of the original 12 members of ASTEC have already been put off, with an accompanying degree of public fuss. The remaining six include the original Chairman, Dr Lewis Matheson, who has been retained in a part-time capacity. The Advisory Group has ensured its own continuing influence by successfully recommending that they be appointed en bloc to the reconstituted Council. Two of the Advisory Group (Dr Matheson and Professor Robert Street, an increasingly influential figure in Australian science politics) were already on the previous Council; the other three included two from the Australian Academy of Science. With at least five members on the new Council, the Academy has the dominant scientific voice. Remaining places on the Council are certain to be filled by people from industry and commerce.

ASTEC's retention and transfer to the Prime Minister's direct responsibility is the clearest signal yet that the Department of Science and its Minister has a major fight on its hands for survival. ASTEC is now in a position to assess the Department of Science's own priorities. The Department is listed as but one of five Federal Departments plus one Agency (CSIRO) with significant research and development budgets which will be "invited to attend all meetings of the Council but without voting rights and without any responsibility for the Council's decisions."

Further, the Minister for Science publicy acknowledged at the ANZAAS Congress in Hobart on May 10 that his Department is under real threat of abolition. He blamed the report of the Science Task Force of the Royal Commission on Australian Government Administration for "doing a disservice to the scientific community in advocating the abolition of the Department". Senator Webster was merely reflecting the understandably critical stance of his Department's Secretary, Sir Hugh Ennor, to the draft of the Task Force's report released in January. Unrepentant, the Task Force's final report to the Royal Commission has stuck to its original recommendations that the operational branches and sections of the Department be distributed around other departments with scientific components. The Royal Commissioner, Dr H. C. Coombs, is now considered likely to accept these recommendations in his report due before the end of June.

Running parallel to the Royal Commission, which had been set up by the Labor Government, is Mr Fraser's own closed inquiry into the structure of the Australian Public Service. Headed by a former public servant, Sir Henry Bland, this committee is sending tremors throughout Canberra. If it runs true to the spirit of its founding father, who likes to be seen as a man of steel, the Bland Committee will recommend (again shortly) that various departments and agencies be amalgamated or some of their functions transferred to the six States of Australia. The Department of Science is a prime target.

In all of these hassles, the Labor Party and its science spokesman, Mr Rex Connor, have maintained a level of public disinterest which has done nothing to enhance their reputation as anything more than the initiators of science policy discussions at a political level.

Among scientists, a sense of *déjà vu* has set in. A dreary and poorly attended symposium on science policy at the ANZAAS Congress was in sharp contrast to lively symposia on the subject at the previous two Congresses held during Labor's reign. The debate has given all the appearances of degeneration into internalised rows about administrative structure (for example, ASTEC versus the Department of Science). Questions of the content of research and development and national priorities for research funds seem, for the moment, to have been put into the "too hard" basket. ☐

IN BRIEF

Nuclear actions

On paper, at least, the world became a safer place last month, when Japan became the 96th nation to ratify the Non-Proliferation Treaty (NPT) while the Soviet Union and the USA signed a treaty limiting the size of single nuclear explosions "for peaceful purposes" to 150 kilotons or less. The US–USSR agreement was officially hailed as a major breakthrough because of its provision for on-site inspection by either party of single explosions over 100 kilotons or multiple explosions below the upper aggregate limit of 1,500 kilotons; this, it is claimed, could clear the way for future on-site monitoring of military test blasts.

But with no satisfactory conclusion to the Strategic Arms Limitation Talks in view, and no provision for on-site inspection in the 1974 threshold agreement on military tests (which forms the major part of the newly completed treaty), liberals regard the achievement sceptically, particularly as 150 kilotons is seen as a meaninglessly high limit. Both the military and non-military treaties are expected to come into force simultaneously following Senate ratification.

Japan's ratification of the NPT, ending a stormy six-year passage through the Diet, came as a result of strong US pressure, according to some Japanese government sources, US interests supply Japan with enriched uranium for her nuclear reactors. Japanese determination to achieve greater nuclear independence is, however, emphasised by its $8,400 million nuclear fuel strategy aimed at securing an annual 21,700 tons of uranium oxide by 1995. Japan hopes to build up 130 million kilowatts of nuclear power in place of petroleum over the next 20 years. By 1980 the proportion of domestic enrichment should have risen from 2 to 33%.

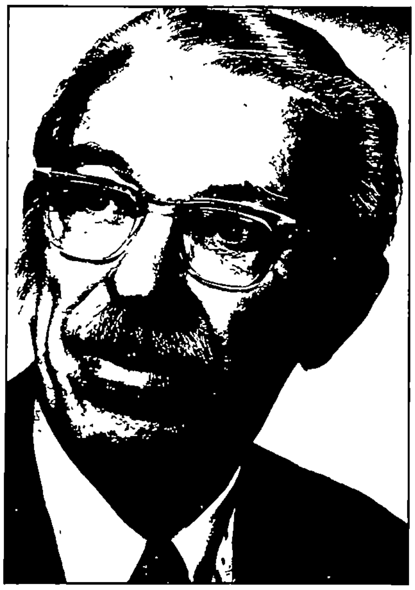
Meanwhile, amid European plans to dump 6,700 of packaged radioactive wastes in 4,500 metres of Atlantic water next month, news broke of the first detected leak of plutonium from waste drums dumped off America over the past 20 years. Plutonium, a carcinogen threat, returns to man through the food chain.

Moves over fishing limits

The agreement reached last week between Britain and Iceland to end the six months' old Third Cod War will itself last six months, during which Britain hopes to resolve two problems largely responsible for its interim character: the question of 200-mile international limits, which the unfinished UN Law of the Sea Conference will discuss further in August; and the matter of an EEC Common Fisheries Policy, on which the Nine seek agreement regarding wider exclusive national limits inside a 200-mile EEC "pond".

Delay on both has complicated the position of Norway as well as of Iceland, and she too may operate the 200-mile limit she seeks in the absence of an EEC agreement. Britain's special difficulty as the manoeuvring amongst the Nine continues is that her reported "target" limit, in the 35–50 mile range, though higher than the current 12-mile exclusive limit, is far less than the 100 miles sought by British trawlermen For all countries, however, the top priority remains the conservation of fish stocks.

Human testing



THOMAS H. JUKES

EXPERIMENTS with human subjects are increasingly being restricted by regulations and decrees. This is probably just as well, as long as the pendulum doesn't swing too far. Vertebrates in general are now regarded with a kindly eye, unless they are considered to be edible. "The more I see of some people—any people—the better I like sea-lions". That is the cry. Experiments with *Escherichia coli* are getting a hard look. Soon we may hear that bacteria have rights, too. After all, the real difference between them and us is a disparity in the length and sequence of DNA molecules.

On the human scene, the California law-makers are pondering a bill (S-2051) saying that various categories of people cannot give "informed consent" to experiments performed on their bodies. Included under this protective umbrella are prisoners. Graduate students are not mentioned; I noted this with some nostalgia, for I am a relict of an age when we assumed risks to gain benefits. Not any more. Let the other guy take the risks. And yet . . . as Charles Best said,

> So now Banting and I rolled up our sleeves I injected him with our extract and he injected me—we had to be sure it wasn't too toxic to be tolerated by human beings.

Someone, in December, 1921, had to be the first to be injected with insulin, so that many who could not, or did not, give "informed consent" would benefit in years to come. The first patients to receive insulin did so without talking to their lawyers about malpractice suits.

It was a romantic era; there were giants in those days. One was Howard Taylor Ricketts, who lifted five lice from a patient with typhus fever, and put them in his pocket in an envelope When Ricketts opened the envelope later, in his laboratory, there were only four lice; the other one had escaped to his body. Ricketts died of typhus fever some days later He was not the last to die of a laboratory infection while working on typhus, before it was brought under control by yolk-sac vaccine, DDT and tetracycline antibiotics. The US Public Health Service, Hamilton, Montana laboratory published a bulletin in the 1930s that was dedicated by its authors to their colleagues who had died of Rocky Mountain spotted fever. The laboratory was conveniently near to a canyon that supplied ticks with a "hot strain" of the infective agent.

Prisoners often gave their informed consent to experiments performed on them. In some cases, their professed motivation was a desire to pay a debt that they thought they owed to society, rather than the hope of special privileges. The soldiers who volunteered to be bitten by mosquitoes that had fed on yellow-fever patients were compensated, if they survived, by sums of $300. The widow of one of them received an annual pension of £1,500.

Most experiments with human subjects involved very little risk. I remember, as a graduate student, drinking a beaker of milk that had been liberally laced with staphlococcus toxin. Nothing happened, and I collected ten dollars Gone are those bad old days; the lawyers and the government are looking after us

correspondence

Selling the Sun

SIR,—Since I am part owner of what I believe to be the only completely privately built and financed house in Britain to incorporate solar central as well as water heating, I would like to present some information which could have a bearing on your editorial (May 20, page 177).

The installation was designed by Stephen Szokolay, who also designed the Milton Keynes House. Central heating is by circulation of warm water through pipes in the floor, to maintain a background warmth; water heating is by a heat exchanger in a conventional domestic tank.

In the absence of a systematic monitoring programme—it being difficult to get any financial support for what most people clearly thought was either a con-trick or the vapourings of a diseased mind—I have estimated that of the approximately 17,000 kWh annual heating demand predicted by the designer, the sun has supplied in the order of 9,000 kWh, possibly more, but certainly not much less, during the first year of operation.

The house is insulated to above-average standards, and the underfloor pipe system ensures that in summer months on very hot days, excess hot water can be pumped through the floor, which thus acts both as heat sink and heat store. If the heat decay curve for the floor slab is flat enough, some residue of surplus summer heat could possibly be retained into autumn.

Electrical immersion heaters and a wood stove are used as back-up heating for sunless days During last winter there were periods of up to 2 weeks when the solar input could only be described as negligible. In the absence of a completely new style of house design, I cannot see how the need for a back-up system is to be avoided.

Which brings us to costs. First, energy costs: my rather crude calculations lead me to conclude that the energy cost of materials over and above what would be required for a conventional fossil-fuel piped heating system (that is, collector panels and glazing, storage tank, anti-freeze, piping, pumps, switches) is in the order of 20–25,000 kWh. The system needs to run trouble-free for at least five or six years in order to pay for itself in energy terms.

Second, raw material costs: several hundred extra kg of assorted metals and a similar amount of glass may seem rather a high price to pay for "free heat". There is no way in which the sun can pay off the costs. Third, financial costs: because the suppliers of components were extremely generous, the final cost of the installation, at about £1,200 (at 1974–75 prices) can be described as a bargain. We must clearly wait rather more than 5–6 years for the sun to pay that off for us.

I present these points for interested readers to ponder. But as one who hates waste, I must say that when the sun comes out and I hear the switches go on, I get a warm glow.

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Birds on the Chagos Bank

SIR,—We share the concern expressed by Messrs. Hirons, Bellamy and Shepherd (*Nature*, **260**, 387; 1976) for conservation in the Chagos archipelago, but we should like to comment on their proposal to exterminate the rats there. The fourteen islets on the western edge of the Chagos bank are only a fraction of over sixty in the whole Chagos archipelago, and over six hundred in the whole Indian Ocean, considered worthy of assessment for conservation by the International Union for the Conservation of Nature (Elliott, H.F.I , *J. mar. biol. Ass. India*, **14**, 578–608; 1974). Many of these are of more scientific importance and more seriously threatened. Before scarce resources are devoted to an attempt to eliminate rats from the two largest of the western Chagos islands—something which is likely to be difficult and costly and has rarely been accomplished even on much smaller and more accessible islets elsewhere—perhaps the need requires to be demonstrated more clearly than has yet been the case in Dr Bellamy's television film about his previous expeditions.

From the information available so far (Bourne, W R.P., *Atoll Res. Bull.*, **149**, 175–207; 1971), there appears to be nothing particularly unusual about the islands in question. While there has been a previous report that Abbott's Booby *Sula abbotti* has been seen at sea in the area, there is an immature plumage of the Masked Booby *Sula dactylatra* which strongly resembles that of adult Abbott's Boobies, and it would be helpful if more details of the birds seen could be provided to confirm their identity. If Abbott's Booby were found nesting in the group, this could be of crucial importance for the species, which is now reduced to about 2,000 pairs threatened by phosphate mining on Christmas Island (Nelson, J.B., *J. mar. biol. Ass. India*, **14**, 643–662; 1974). On the other hand, although rats have reached Christmas Island they have not harmed the tree-nesting boobies there yet, although the birds might be more vulnerable in the lower vegetation of the Chagos group. The case for regarding the western Chagos islands as a potential refuge for this remarkable booby has still to be proved.

The other seabirds frequenting the Chagos group are all widespread species Despite statements in the film that the Masked Boobies show unusual features, the specimen from the Chagos group deposited in the British Museum (Natural History) appears to us to fall within the normal range of variation for the western Indian Ocean. Together with the smaller seabirds its numbers seem more likely to be limited by conditions at sea than on land, and it seems likely the birds can still find ample breeding-sites on the undisturbed, rat-free outer islets, while the few landbirds now present in the archipelago appear to be introduced. It therefore seems questionable whether the rats are causing any more harm to the birds than to the vegetation.

While we yield to nobody in our concern at the harm caused by introduced mammals on islands (Bourne, W.R.P., *New Scientist*, **67**, 422–425; 1975), we feel that it might be more useful if before they try to exterminate the rats Dr Bellamy and his colleagues were to dispose of the donkeys left on Diego Garcia, Ile du Coin and Ile Boddam elsewhere in the Chagos group at the time of their enforced evacuation in the early 1970s (Curtis, W.F., *Sea Swallow*, **25**, 11–13; 1976). These seem likely to present a really serious threat to the regenerating natural vegetation if they are left to increase. It would also be interesting to know what arrangements our authorities have made to maintain continuing scientific surveillance of these potentially important nature reserves now that they have evicted their human inhabitants.

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news and views

Transient galactic radio and X-ray sources

from F. G. Smith

DETAILED correlation between the radio and the X-ray emission of celestial sources is hardly to be expected in view of the different radiation mechanisms involved at such widely different parts of the spectrum. A general correlation might, however, be expected from the association both of X-ray and radio emission with unusually energetic objects, some of which are variable. Some such are now turning up.

Many galactic X-ray sources are variable, but rather few are also radio sources. A large part of a single issue of *Nature* was recently devoted to the source Cyg X-3, which has given a series of very large radio outbursts which were not related in detail to its X-ray variations. Another example, A0620-00, gave an X-ray outburst which again was detected at a wide range of radio frequencies (Little *et al.*, *Nature*, **261**, 113; 1976); here the radio variations followed fairly closely the X-ray flux. The problem in interpreting the radio emissions is to extend observations to low enough frequencies to establish the spectrum, and in particular to look for the self-absorption typical of synchrotron radiation. Observationally, this means detecting a very weak source among the multitude of others, and finding a sufficiently accurate position for the identification with the X-ray source despite the problems of position-finding at long radio wavelengths.

The transient radio source reported by Davies *et al.* (page 476 of this issue of *Nature*) was found during a study of the galactic centre, using the high resolution interferometers at Jodrell Bank, operating at 960 MHz and 408 MHz. The source was detectable for one day only, but fortunately it was sufficiently near a known radio source for a position to be found to within 3 arc s. The position fits well with that of the X-ray source A1742-28, and incidentally improves the positional accuracy of that source by over 500 times (expressed as an area).

The meagre spectral information given by this one result suggests that this source is more like A0620-00 than Cyg X-3 It would be useful to find it optically, but there is not much hope of this in the crowded and obscured galactic centre region. The accurate position will, however, make the search worth trying.

Many X-ray sources are known to be members of close binary systems, in which the atmosphere of a compact star is heated by the transfer of material from a giant. The tantalising glimpses which we now have of the radio and X-ray variables suggest that another type is involved, in which the energy source is more sporadic In the centres of globular clusters, for example, there might be collisions or near-collisions between stars, giving rise both to heated dense atmospheres for the X rays and much more extended atmospheres, probably containing magnetic fields as well as charged particles, which give the radio emission. The proximity of this source to the galactic centre will encourauge thought on such lines. ☐

Space probes *versus* comets

from David W. Hughes

THE fringes of the Solar System obviously contain mass, any comet with a period greater than 300 years spending a considerable fraction of its life beyond the orbit of Pluto. Öort (*Bull Astron. Inst. Neth.*, **11**, 91; 1950) went so far as to propose the existence of an enormous cloud of comets with aphelias some tens of thousands of a.u. from the Sun. Detecting this mass is the problem. In 1972 Axford proposed that the cometary belt could be detected by observing its gravitational effect on a spacecraft which is headed out of the Solar System. A. P. Boss and S. J. Peale (Department of Physics, University of California) show, however, that the secular perturbations of the orbits of long-period comets are currently three orders of magnitude more sensitive than spacecraft for detecting an unseen distribution of cometary or other masses near the outer regions of the Solar System (*Icarus*, **27**, 119; 1976).

The authors produce specific models for the mass distribution and then, using a numerical integral computation, find the acceleration that this mass distribution gives to a space probe as it moves through the Solar System. The velocity of the spacecraft along the line of sight can be easily obtained from the Doppler shift in the frequency of the transmitted radio signal. A one minute integration of the Doppler data enables the line of sight velocity to be measured to an accuracy of ±0.03 cm s^{-1}. Temporal variations in this velocity give the acceleration. Most spacecraft, including the deep space probes Pioneer and Mariner, suffer from outgassing and other variable effects which produce stray accelerations. These can be as high as 10^{-7} cm s^{-2} and random in direction This uncertainty leads to a total minimum detectable cometary-cloud mass of the order of a thousand Earth masses.

Now Halley's comet has a period of about 76 years and an aphelia at about 35 a.u. Hamid, Marsden and

Whipple (*Astr. J.*, **73**, 729; 1968) analysed the orbits calculated from the 1835 November return and the 1909 October return and found that the argument of perihelion had increased by 1.039°, the ascending node by 1.045° and the inclination had decreased by 0.041° during this period. These orbital changes could have been caused by the gravitational effect of a hypothetical ring of matter in the ecliptic plane of the Solar System, this ring having a mass of 0.5 Earth masses and a radius of 40 a.u., or alternatively a mass of 1.3 Earth masses at a radius of 50 a.u., or a larger mass still at a larger radius. It now seems certain, however, that the long term motion of Halley is also significantly affected by "non-gravitational" forces. These are, in the main, caused by the rotation of the cometary nucleus coupled with the monodirectional incident radiation flux leading to an asymmetric efflux of gas and a resultant force in the plane of the orbit.

The problem is that the exact magnitude of the non-gravitational force effect is unknown, the results given above for the possible mass of a comet belt thus becoming upper limits.

Comets still have, though, an enormous (approximately one thousand times) sensitivity advantage over space probes when it comes to detecting hidden distributed mass in the Solar System, even though their effectiveness is decreased by unknown non-gravitational forces. The only way a space probe could overcome this advantage is by being especially designed to be clean and gas free and by being solely dedicated to the specific task of mass detection. ☐

Phytochrome and gibberellins

from John L. Stoddart

When Jack watched the beanstalk climbing into the heavens he was probably witnessing one of the earliest recorded instances of gibberellin-stimulated extension growth and many plant physiologists have since wished that he could have found some way to bequeath his unique mutant to posterity.

Gibberellins are amongst the most potent of the naturally occurring plant growth regulators and exert maximal activity in most tissues when present in sub-microgram quantities. In the 38 years since the first crystalline isolation of a gibberellin by Yabuta and Sumiki (*J. agric. Chem. Soc. Japan*, **14**, 1526; 1938) there has been a steady, and more recently rapid, increase in our knowledge of the structural variations which can occur (almost 50 gibberellins have been characterised to date) and the ways in which plants synthesise or degrade these compounds. On the other hand, although gibberellins are now used extensively as malting accelerators in the brewing industry and as fruit-setting agents for seedless grapes, we are still largely ignorant of the precise means whereby these highly active compounds influence plant growth.

In many cases there are clear relationships between light, expressed either as photoperiod or spectral composition, and responsiveness to gibberellins with some of the most intriguing observations being related to red/far-red (phytochrome dependent) light treatments. The nature of the relationship between gibberellins and the photomorphogenic pigment, phytochrome, in cereals has been examined in a recent series of publications on gibberellins in the plastids of dark-grown leaves (etioplasts).

When dark-grown cereal leaves are exposed to red light (660 nm) for as little as 15 min, very large increases occur in the extractable gibberellin content (Reid *et al.*, *Nature*, **217**, 580; 1968) and this response is known to require active RNA and protein synthesis. Up to 60% of the total gibberellin content of the leaf can be accounted for in terms of the biological activity contained in the chloroplast fraction (Stoddart, *Planta* (*Berl.*),**81**, 106; 1968) and thus it is reasonable to look to the plastid for light-mediated effects on gibberellin activity. Using isolated etioplast preparations, Cooke and Saunders (*Planta* (*Berl.*), **123**, 299; 1975) have shown that the red light effect on extractable biological activity could be solely attributed to the plastid and that no comparable increase occurred in other cell fractions, although these contained measurable amounts of gibberellin in both light and dark. Phytochrome was detected in the etioplast preparations (Cooke *et al.*, *Planta* (*Berl.*), **124**, 319, 1975) and the increased biological activity was attributable to changes in at least two gibberellin-like components. The latter term is used when describing activity measurements based upon bioassays and where the active compound has not been chemically characterised.

These findings have been confirmed recently by Evans and Smith (*Proc. natn Acad. Sci. U.S.A.*, **73**, 138; 1976) using carefully authenticated etioplasts prepared by similar procedures. Their results strengthened the causal nature of the co-occurrence of gibberellins and phytochrome by demonstrating that the enhancement of biological activity was far-red (730 nm) reversible.

Both groups have suggested that phytochrome could act by regulating the permeability of the plastid envelope to gibberellins, with the inference that this allows access to cytoplasmic or wall-bound action sites: an interpretation supported by the recent isolation of enriched envelope preparations containing small quantities of phytochrome (Evans and Smith, *Nature*, **259**, 323; 1976). The pigment content of this fraction was low when compared with that of the original homogenate (<1%) but no other etioplast subfractions contained measurable amounts. It is possible, however, that the pattern of associations could change after red irradiation. How then might envelope permeability changes account for the observed increase in plastid gibberellin activity after 660 nm irradiation? There are two possibilities. Evans and Smith propose that the first response is an efflux of gibberellin resulting in internal depletion which, in turn, stimulates biosynthesis by way of a feedback activation mechanism. There is evidence that chloroplasts do indeed have a gibberellin biosynthetic capacity (Railton and Reid, *Plant Sci. Lett.*, **3**, 303, 1974) and Cooke *et al.*, showed that gibberellin biosynthesis inhibitors greatly reduced the red light response. The available inhibitory compounds are, however, of suspect specificity and some are known to have more general effects on protein synthesis.

An alternative explanation, initially advanced by Cooke and Saunders, is that increased biological activity reflects release from a 'bound' form (attachment to either proteins or membranes) within the plastid and, in this case, effects upon envelope permeability would be a secondary correlated process facilitating efflux of the released gibberellin. Data presented by Evans and Smith showing that ultrasonic treatment of unirradiated etioplast preparations can give a three-fold increase in extractable biological activity are possible support for this mechanism. In addition to rupturing the envelope, such treatments would be expected to solubilise material associated with lipoprotein or membranes. The rapidity of the red light effect is also consistent with the binding hypothesis.

Localisation of phytochrome in the envelope indicates that this may be a primary site of action but it is still uncertain whether the increased extractable gibberellin-like activity is produced by *de novo* synthesis using stored precursors or is the result of release from a bound pool. Further studies on gibberellin localisation and synthetic capacity in etioplasts are needed before this aspect can be resolved. It seems that we may now be in sight of a possible resolution of the apparent discrepancy inherent in the observation that applied gibberellin will give a massive growth stimulation in tissues containing quantities of extractable activity sufficient to sustain the maximum potential growth rate There could also be an acceptable explanation for the interchangeability of red light and exogenous gibberellin in the control of leaf unrolling in dark-grown cereals (Loveys and Wareing, *Planta (Berl.)*, **98,** 117; 1971).

Compartmentalisation of endogenous plant growth regulators coupled with environmentally-mediated release mechanisms offers an attractive solution to these, and many other, problems of plant development. The prospects are exciting and there are indications that the existence of such a system for leaf gibberellins may soon be unequivocally established. ☐

Polyamines and growth control

from Robert Shields

ALTHOUGH it has been known for some time that polyamine levels alter with changing rates of cell proliferation, the precise role (if any) that these molecules have in cell regulation is still a mystery.

The first step in the synthesis of polyamines is the decarboxylation of ornithine to form putrescine by ornithine decarboxylase (ODC) which is the rate limiting enzyme for polyamine synthesis This enzyme is somewhat unusual in that its activity in a given tissue can vary by more than 20-fold in different conditions and it must also hold the world record for the fastest vanishing mammalian enzyme activity with a half life that can be as short as five minutes in some conditions. It is then hardly surprising that ODC has attracted so much attention. The second enzyme of the pathway, S-adenosyl methionine decarboxylase (SAM decarboxylase) takes part in the conversion of putrescine to spermidine and the sequence is completed with the conversion of spermidine to spermine. Increased levels of ODC and SAM decarboxylase invariably accompany increased cell proliferation and indeed the levels of these enzymes may be very closely correlated with the specific growth rate of certain cells (Heby *et al*, *J. Cell Physiol*, **86**, 511; 1975). Presumably it is not the levels of these enzymes themselves that may regulate growth but rather the polyamines they produce Careful analysis of polyamine concentrations and growth rate show that levels of spermidine seem to be closely linked with cell proliferation rates whereas putrescine and spermine show little or no correlation (Heby *et al*, *op cit*) No amount of correlative evidence can prove a regulatory role for polyamines however, clearly a more direct approach is necessary.

If polyamines are growth regulatory then interfering with their synthesis by use of enzyme inhibitors should interfere with cell growth. And this is what happens; cell division proceeds more slowly and in some conditions may cease altogether. Two types of inhibitors have been used; one is the anti-leukaemic agent MGBG which inhibits SAM decarboxylase and the others are various ornithine analogues which inhibit ODC Using MGBG Fillingame and Morris were able to block the increases in spermidine and spermine that are normally observed after stimulation of lymphocytes by concanavalin A (con A) The increased synthesis and processing of RNA and synthesis of protein brought about by con A continued unimpeded however (*Biochemistry*, **12**, 4479; 1973).

This important observation shows that increases in spermidine and spermine are not necessary for increases in protein and RNA synthesis although a requirement for certain basal levels of polyamines in macromolecular synthesis cannot be ruled out Although MGBG had no effect on the increased synthesis of RNA and protein the drug could partially inhibit DNA synthesis. The inhibition was somewhat unusual in that the number of lymphocytes that entered DNA synthesis after con A stimulation was unaltered but progress through S phase to mitosis was impaired (Fillingame *et al., Proc natn. Acad Sci U.S.A.*, **72**, 4042, 1975) These results suggested that increased levels of spermine or spermidine might be necessary for normal progress from DNA synthesis to cell division. One possible objection to these experiments is that blocking SAM decarboxylase with MGBG leads to elevation of putrescine levels which could fulfil the functions of spermidine and spermine sufficiently well for increased RNA and protein synthesis but not for DNA synthesis to occur If this were the case it might be expected that blocking putrescine formation and hence the synthesis of spermidine and spermine might have more profound effects than those obtained with MGBG.

Precisely this line of approach is taken in recently reported experiments using α-methyl ornithine to inhibit ODC and hence polyamine synthesis (Mamont *et al*, *Proc natn Acad Sci U S A.*, **173**, 1626; 1976). These workers used a suspension culture of a hepatoma cell line (HTC) whose growth rate is slowed at high cell densities, diluting the culture leads to a rise in ODC and polyamine levels followed by increased rates of DNA synthesis and cell division. If the culture is diluted in the presence of α-methyl ornithine, the cells enter DNA synthesis and divide normally but subsequent rounds of DNA synthesis and cell division are inhibited and cell proliferation ceases. Examination of polyamine levels after culture dilution shows that putrescine concentrations follow the activity of ODC (McCann *et al*, *Biochem, biophys Res. Commun*, **64**, 336, 1975), peaking some time before the increase in DNA synthesis. In the presence of α-methyl ornithine putrescine concentrations fall very rapidly but no inhibition of DNA synthesis occurs until the spermidine levels have dropped significantly by which time the cells would normally have entered the second wave of cell division. The failure of the cells to enter this second round of cell division can be directly related to low spermidine levels as spermine concentrations remain relatively unchanged. Taken together with the results from lymphocytes and the close correlation between spermidine levels and growth rate it seems that of the polyamines only spermidine may play a part in DNA synthesis and cell division. A certain basal level of polyamines may be necessary for normal growth, however, and experiments using inhibitors of polyamine synthesis and the selection of cell mutants with altered polyamine metabolism may shed some light on the normal functions of these curious compounds. ☐

NATURE

A hundred years ago

THE comparatively infrequent employment of electric light, considering the great success achieved in its production, would at first sight appear to be due to something in the application of the electricity itself. It has been repeatedly and satisfactorily proved that a continuous and powerful light can be produced by electricity, and the question naturally arises, Why is it not more frequently employed for practical purposes?

Unquestionably the first experiments with electric light were not successful, but this is generally the case with new inventions. Unfortunately, however, a feeling seems to have arisen directly against the application of electricity for lighting purposes, or at any rate against the employment of the existing apparatus in the hope that more perfect may soon be invented.

from *Nature*, **14**, 133–144, June 8, 1876

Reactions to nuclear anti-analogue states

from P. E. Hodgson

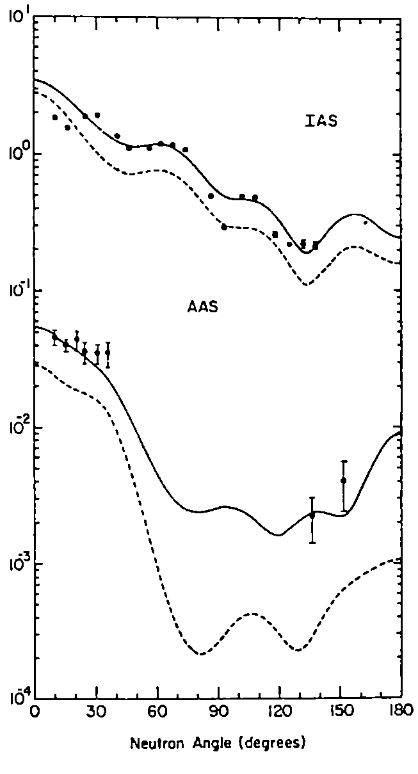
REACTIONS to isobaric analogue states have been extensively studied ever since their discovery by Anderson and Wong in 1962, and have yielded much useful information on nuclear structure and on the nucleon optical potential (*Nature*, **247**, 179; 1974). More recently, it has proved possible to detect reactions to anti-analogue states, and analyses of their cross sections are giving interesting results.

The simplest reaction to these states is the (p,n) reaction, that adds a proton and removes a neutron from the ground state of the target nucleus. The final nucleus thus has the same number of nucleons as the target nucleus and essentially the same mass; this is why the final states are called 'isobaric'. At the same time the charge has been increased by one, and the ways of doing this can most easily be described using the isobaric spin formalism, which is exactly similar to the corresponding formalism for ordinary spin. Each nuclear state has an isobaric spin, with three components like a spin vector. The third component T_3 is directly related to the nuclear charge, $T_3=\frac{1}{2}(N-Z)$, where N and Z are the numbers of neutrons and protons in the nucleus. All the states of a nucleus thus have the same value of T_3, but they can have any total isobaric spin equal to or greater than T_3, since no vector can be shorter than any one of its components.

The ground states of nuclei nearly always have $T=T_3$, so when we increase the charge by one by a (p,n) reaction we reduce T_3 by one, since by convention a neutron has $T_3=\frac{1}{2}$ and a proton has $T_3=-\frac{1}{2}$. The final states can thus have a total isobaric spin of T or $T-1$. The state with isobaric spin T is the isobaric analogue state, and apart from small electromagnetic effects it has exactly the same structure as the target ground state, except of course for the reduced charge. All the nucleons are coupled together in the same way, and we can say that the only difference is that one isobaric spin vector has been flipped so that its third component is $-\frac{1}{2}$ instead of $\frac{1}{2}$. Quantum mechanically the states have a very high overlap, and so reactions from one to the other take place easily, and it is their high cross sections that enable them to be detected even though many other reactions were also taking place.

The anti-analogue state with isobaric spin $T-1$ is just the opposite; quantum mechanically its structure is nearly orthogonal to that of the ground state of the target. Reactions to this state are thus very difficult, and can only take place at all because of small differences in structure from complete orthogonality. Unlike the analogue state reactions, the anti-analogue state reactions are sensitive to small components of the wavefunctions, and they are thus difficult to describe theoretically as well as being difficult to detect due to their low cross sections.

Early work on anti-analogue state reactions used the (^{3}He,t) reaction, which also has the net effect of adding a proton and removing a neutron. Calculations of the cross section of the reaction on ^{40}Ar by Schaeffer and Bertsch (*Phys. Lett.*, **38B**, 159; 1972) showed that the direct component can only account for a very small fraction of the observed cross section, but that a reasonable fit can be obtained by assuming that the reaction takes place mainly by a two-step process in which a (^{3}He, α) reaction is rapidly followed by a (α, t) reaction. Similar results were obtained for the (^{3}He,t) reaction on ^{48}Ca.



Differential cross sections of the ^{40}Ar(p,n)^{40}K reaction at 24 MeV to the isobaric analogue and anti-analogue states compared with distorted wave calculations assuming that the reactions take place in one step. The dashed curves show the result of the direct transition only and the solid curves the result of including the knock-on exchange contributions. The isobaric analogue state cross sections were obtained by Bentley and colleagues (*Phys Rev. Lett.*, **27**, 1081; 1971).

Studies have now been made of the (p,n) reaction to the anti-analogue state of ^{40}Ar by Galonsky and colleagues of Michigan State University (*Phys. Rev. Lett.*, **35**, 1208; 1975). They used 24 MeV protons, and were able to detect the very small cross sections of the anti-analogue state reaction using a large liquid scintillator and a time-of-flight spectrometer. As shown in the figure, these cross sections are only about 1% of the corresponding analogue state ones.

They analysed these data using the distorted wave theory assuming that the reaction takes place on one step, and including both the direct and the knock-on exchange contributions. The dashed curves in the figure are for the direct process only and the solid curves show the result of including exchange. The parameters of the distorting potentials and of the effective nucleon–nucleon interaction were all taken from previous work.

It is clear from the figure that this calculation gives cross sections in very good agreement with the experimental data, showing that at least in this case it is not necessary to take into account the possibility of two-step reactions in which the incident and outgoing particles are composite, for then the exchange processes are expected to be more likely than the direct.

The relative probabilities of one-step and multi-step processes is a very interesting question, and it is likely that further studies of reactions to anti-analogue state will provide important information. They should also tell us more about the smaller components of the nuclear wavefunctions. ☐

Mammalian DNA repair

from a Correspondent

The Second International Workshop on DNA Repair Mechanisms in Mammalian Cells was held at Nordwijkerhout, the Netherlands, on May 2–5.

ALL cellular systems that have been examined possess repair mechanisms for dealing with lesions introduced into DNA. The general purpose of investigations on mammalian DNA repair and of the Second International Workshop on DNA Repair Mechanisms in Mammalian Cells has been to understand how damage in cellular DNA is recognised and removed—or alternatively bypassed—and the effect of the processes involved (that is, mutation or cancer).

The inability to carry out normal repair or DNA metabolism is associated with several human diseases, which were summarised by J. German III (New York Blood Center). Among the diseases studied extensively are xeroderma pigmentosum (XP) and ataxia telangiectasia (AT), both extremely rare. Cells from affected individuals lack the first step in removing lesions induced in their DNA by ultraviolet light or ionising radiation, respectively, which correlates well with the sensitivity of these individuals to sunlight or ionising radiation. There are now five complementation groups for XP and at least two for AT, based largely on the cell fusion studies of D. Bootsma (Erasmus University, Rotterdam), P Lohman (Medical Biological Laboratories, Rijswijk) and M. Paterson (Atomic Energy of Canada, Chalk River) Although XP cells lack the ability to excise ultraviolet-light-induced pyrimidine dimers from their DNA *in vivo*, the excising function is still present. As shown by E. Friedberg (Stanford University Medical Center), the dimers are removed from "naked" DNA added to crude extracts of these cells. Since extracts of cells from normal individuals can carry out DNA repair on chromatin from irradiated XP cells and XP extracts cannot, it was concluded that repair must be considered at the level of chromosome structure as well.

A DNA unwinding protein may be important for repair at the chromosome level. A. Huang (Duke University Medical Center) has isolated such a protein from lymphocytes of patients with chronic lymphocytic leukaemia. It enhances the first step in pyrimidine dimer removal, by repair enzymes, causes a decondensation of chromatin, and is present at about three to five times the concentration observed for normal cells. Furthermore, there is considerably more repair synthesis after ultraviolet irradiation of cells from patients with this leukaemia compared with normal cells.

Another type of repair has been identified, first in bacteria and then in mammalian cells, which allows semiconservative replication past pyrimidine dimers in DNA. Frequently gaps are left in the DNA and these are filled in later either by *de novo* synthesis or, based on the work of R. Meneghini (University of San Paulo) and A. Lehmann (MRC Mutation Unit, University of Sussex), by possible exchange processes which can result in dimers appearing in DNA synthesised after irradiation. Approximately 20% of the dimers appear in new DNA. The reassortment may correlate with the high frequency of sister chromatid exchanges which result after ultraviolet irradiation of cells from XP patients as observed by de Weerd-Kastelein (Erasmus University, Rotterdam). Molecular evidence for recombination of mammalian DNA was presented by P. Moore (National Institute for Medical Research, London). He reported that a small proportion of DNA recovered from cells treated with mitomycin C (a carcinogen that in fungi is also a potent recombinogen) has the hybrid intermediate characteristic of DNA undergoing recombination The importance of being able to carry out genetic recombination was emphasised by the work with lower eukaryotes summarised by M Resnick (National Institute of Medical Research, London). Evidence was presented that haploid organisms, with only one copy of their genome, are much more sensitive to ionising radiation than those which are diploid (including mammalian cells) or haploid but with a duplicated genome; he suggested that this may be due to the capacity to undergo recombination.

Some of the gaps which occur in newly synthesised DNA after exposure to ultraviolet light have been suggested by S Sedgwick (Laboratoire d'Enzymologie, Gif-sur-Yvette) to be the sites of very high levels of mutation An inducible protein which may be error prone has been proposed by M. Radman (University of Brussels) to be part of an "SOS" repair system in bacteria Evidence from his laboratory indicates that a protein is induced in a mutant strain of *E. coli* that promotes misincorporation of nucleotides into DNA *in vitro*.

Prompted by the work in bacteria, investigations have begun on the appearance of inducible proteins after mutagen treatment of animals or mammalian cells. R. Hart (Ohio State University Hospital, Columbus) has identified a protein fraction from whole animals and from cells in culture that appears after treatment with ultraviolet light or target carcinogens (those which act on specific tissues; for example, 3-methyl chloranthracene acts specifically on lung tissue). This protein is similar to the "protein x" from bacteria in that it has a molecular weight of 40,000 and is inducible by mutagen treatment

The lesions induced by irradiation and mutagen treatment were the subject of considerable discussion. Many studies have focused on assaying the repair of single types of damage such as pyrimidine dimers produced by ultraviolet light or breaks in DNA induced by ionising radiation. P Cerutti (University of Florida, Gainesville) summarised observations from various laboratories which have shown that several types of lesions may be produced by a given agent It was apparent that considerable caution should be used when drawing conclusions about the repair of cellular DNA based on the changes in only a single category of lesions. □

High resolution NMR of glasses

from Paul Calvert

CURRENT attempts to produce high resolution spectra of solid polymers are among the most sophisticated and, to the outsider, incomprehensible experiments in polymer science Results from this technique are now being used to explain results from some of the crudest measurements, namely impact strength measurements. The development of high resolution NMR for isotropic solids means that the motion of any carbon atom or group in a polymer can be measured as a function of frequency and temperature. This is a considerable advance over wide line NMR which cannot distinguish which groups are moving and should tell us a great deal about the structure and deformation of polymers.

High resolution NMR spectroscopy of solids has seemed imminent since Pulse Fourier Transform (PFT) spectrometers became widely available around 1971. Using PFT, spectra from ^{13}C nuclei in natural abundance could be obtained which were well resolved even for quite viscous samples such as liquid polymers, gels and rubbers (for example see Schaeffer, *Macromolecules*, **4**, 110; 1971; and **5**, 427; 1972). In solids, interactions between neighbouring carbon and hydrogen nuclear magnetic dipoles are not averaged out by molecular motion and broaden the spectral lines into oblivion.

One way to avoid this is to use a very strong hydrogen decoupling field to magnetically stir up the protons thoroughly so that there is no net interaction with the carbons. Things may be improved further by using cross polarisation whereby rather than irradiating the carbons directly they are driven by setting the protons spinning at the appropriate frequency and using the internal field from them to drive the carbons (Pines, Gibby and Waugh, *J. chem. Phys.*, **56**, 1776; 1972; and **59**, 569; 1973). For most polymers the end results are disappointing; lines can be seen but are broad enough to overlap This is mainly due to chemical shift anisotropy, the fact that interactions within the molecule make the line position depend on the orientation of the molecule to the magnetic field and with no molecular motion there is no averaging.

An alternative method to narrow the lines is to spin the sample at the "magic angle" 54.7° to the field. At this angle $3\cos^2\theta-1$ is zero and dipolar couplings and chemical shift anisotropies are averaged out. Schneider and coworkers in Prague report the application of this method to polyethylene (*Macromolecules*, **5**, 120, 125; 1970). A spin rate of up to 12 kHz can be produced using an air jet to drive a sample in the shape of a turbine, but for complete narrowing 50 kHz would be needed.

J. Schaeffer of Monsanto, St Louis has described a combination of these two techniques, using strong decoupling and cross polarisation to remove the high frequency part of the interactions and spinning at 3 kHz to remove what was left. The first published results (*Macromolecules*, **8**, 291; 1975) were not overwhelming but those presented at the American Physical Society meeting in Atlanta last month are very impressive (see *Bull. Am. Phys. Soc. Series II*, **21**, 443; 1976). Spectra with a resolution of 2 p.p.m., comparable with those of solutions, were shown for a range of glassy synthetic polymers, ebony and ivory though the last was not resolved as the spectrum is too complex.

Measurement of several relaxation rates associated with the NMR experiment provides information about molecular motions involving each of the carbon atoms in the MHz, kHz and Hz regions. Before, this was only available for the structure as a whole (for a recent review of broad line NMR see McBrierty, *Polymer*, **15**, 503; 1974). This will have a marked effect on our understanding of the glass transition and those secondary transitions in glasses which involve cooperative molecular motions.

In contrast to the sophisticated and expensive NMR apparatus, impact strength (toughness) is usually measured as the energy absorbed when a swinging hammer breaks a sample (a hundred dollars would buy a good one). The fracture process could be characterised by a time of about 100 μs, that is molecular motions at a frequency of 10 kHz. There have been many attempts to correlate this important property with structure and molecular motion but none is very convincing.

Vincent (*Polymer*, **15**, 111; 1974) has demonstrated a correlation of impact strength with dynamic modulus and mechanical loss but only by dividing the impact behaviour into four categories rather than using numerical data. One case that particularly demands explanation is polycarbonate, an exceptionally tough glassy polymer which loses its toughness on annealing. Schaeffer showed at the Atlanta meeting that for seven glassy polymers impact strength increased monotonically with the ratio of NMR relaxation time for main chain carbons at low frequency (T_{CH}) to that at around 10 kHz ($T_{1\rho}$). In other words, if a molecule can relax easily at 10 kHz it has a high impact strength. Polycarbonate had the highest value of $T_{\mathrm{CH}}/T_{1\rho}$ and this decreases by 2½ times on annealing. Discussion of the connection between these motions and impact strength is still at the hand waving stage, but it is suggested that the plastic work of fracture which accounts for the energy absorbed on impact depends on slip between chains which in turn depends on the inter- and intra-chain interactions that govern the motions associated with $T_{1\rho}$. ☐

Predicting volcanic eruptions

from Peter J. Smith

A DISTINGUISHED British volcanologist recently claimed in a radio broadcast that most volcanic eruptions could be predicted if only adequate funds were to be made available for monitoring equipment. But although the prediction of volcanic activity may be easier than the prediction of earthquakes, knowledge of eruptive precursors is still far from complete. Moreover, as Weaver and Malone (*Geophys. Res Lett.*, **3**, 197; 1976) now point out, even in existing types of data there may be more than meets the eye, especially where glacier-clad volcanoes are concerned.

The phenomenon that Weaver and Malone have in mind is seismic events of low frequency (predominant frequency content less than 5 Hz). Such events have been observed for a number of years in Japan where Minakami *et al.* (*Bull. Earthquake Res. Inst.*, **47**, 893; 1969) termed them type B events. They usually occur within 1 km of a volcanic crater and are apparently associated with eruptive and pre-eruptive processes, for in some cases they have been observed to increase in frequency both before and during actual eruptions. This has led to their being used to predict eruptions from some volcanoes, even though their precise origins are obscure.

Type B events have now been recorded in the vicinity of volcanoes in several parts of the world outside Japan, including Alaska, Indonesia and Central America. Apparently identical events have also been observed near quiescent volcanoes in the Cascade Range of Washington state (see, for example, Unger and Decker, *Bull. Seismol. Soc. Amer.*, **60**, 2023; 1970). Because of the obvious potential of such events for eruption prediction, Weaver and Malone have carried out a more thorough study of those associated with Mt Saint Helens, an historically active stratovolcano in the Cascade Range.

Of the thousands of low frequency events recorded, only a few hundred have yet been analysed in any detail. But all were found to have occurred on the glacier-clad north-eastern side of the mountain and all had very shallow depths (<200 m). In these respects, as well as in waveform characteristics, they were therefore easily distinguishable from the deeper (1–3 km) and much less frequent tectonic earthquakes. From this and related evidence, Weaver and Malone conclude that there is a 'compelling' case for attributing the low frequency events not directly to volcanic activity but to glacial movement ('glacier noise'). Indeed, two events studied in particular detail were found to have occurred at a depth of 45±20 m, which is roughly the thickness of the ice in the areas concerned.

If Weaver and Malone are right, there are clearly going to be problems in using type B events for eruption prediction, at least for Mt Saint Helens. But Mt Saint Helens is quiescent, and so the question of prediction is presumably not urgent. By contrast, Mauna Loa, the largest of the five volcanoes making up the island of Hawaii, is in a quite different position.

Mauna Loa has been created by repeated eruptions over several hundred thousand years and during the past century or so has erupted at irregular intervals averaging 3–4 yr. From 1950 to 1975, however, it never erupted at all, although this 25-yr period of dormancy, the longest in the volcano's recorded history, was broken in July 1975. The 1975 eruption was small by Mauna Loa's standards, producing only 3×10^7 m^3 of lava. But according to US Geological Survey scientists it was highly significant, for historically there has been a cyclic pattern of activity comprising two small summit eruptions followed by a large flank eruption.

Assuming this pattern to be repeated, there is now a specific prediction for the second brief summit and major flank eruptions to take place before July 1978. Moreover, seismic data suggest that the flank eruption will occur along the north-east rift. In this case there will be some threat to Hilo, the island's principal city, which lies about 50 km away but in the direction of natural drainage channels which could guide the lava. However, there are contingency plans to redirect the lava if necessary. ☐

review article

Simple mathematical models with very complicated dynamics

Robert M. May*

First-order difference equations arise in many contexts in the biological, economic and social sciences. Such equations, even though simple and deterministic, can exhibit a surprising array of dynamical behaviour, from stable points, to a bifurcating hierarchy of stable cycles, to apparently random fluctuations. There are consequently many fascinating problems, some concerned with delicate mathematical aspects of the fine structure of the trajectories, and some concerned with the practical implications and applications. This is an interpretive review of them.

THERE are many situations, in many disciplines, which can be described, at least to a crude first approximation, by a simple first-order difference equation. Studies of the dynamical properties of such models usually consist of finding constant equilibrium solutions, and then conducting a linearised analysis to determine their stability with respect to small disturbances: explicitly nonlinear dynamical features are usually not considered.

Recent studies have, however, shown that the very simplest nonlinear difference equations can possess an extraordinarily rich spectrum of dynamical behaviour, from stable points, through cascades of stable cycles, to a regime in which the behaviour (although fully deterministic) is in many respects "chaotic", or indistinguishable from the sample function of a random process.

This review article has several aims.

First, although the main features of these nonlinear phenomena have been discovered and independently rediscovered by several people, I know of no source where all the main results are collected together. I have therefore tried to give such a synoptic account. This is done in a brief and descriptive way, and includes some new material: the detailed mathematical proofs are to be found in the technical literature, to which signposts are given.

Second, I indicate some of the interesting mathematical questions which do not seem to be fully resolved. Some of these problems are of a practical kind, to do with providing a probabilistic description for trajectories which seem random, even though their underlying structure is deterministic. Other problems are of intrinsic mathematical interest, and treat such things as the pathology of the bifurcation structure, or the truly random behaviour, that can arise when the nonlinear function $F(X)$ of equation (1) is not analytical. One aim here is to stimulate research on these questions, particularly on the empirical questions which relate to processing data.

Third, consideration is given to some fields where these notions may find practical application. Such applications range from the abstractly metaphorical (where, for example, the transition from a stable point to "chaos" serves as a metaphor for the onset of turbulence in a fluid), to models for the dynamic behaviour of biological populations (where one can seek to use field or laboratory data to estimate the values of the parameters in the difference equation).

Fourth, there is a very brief review of the literature pertaining to the way this spectrum of behaviour—stable points, stable cycles, chaos—can arise in second or higher order difference equations (that is, two or more dimensions; two or more interacting species), where the onset of chaos usually requires less severe nonlinearities. Differential equations are also surveyed in this light; it seems that a three-dimensional system of first-order ordinary differential equations is required for the manifestation of chaotic behaviour.

The review ends with an evangelical plea for the introduction of these difference equations into elementary mathematics courses, so that students' intuition may be enriched by seeing the wild things that simple nonlinear equations can do.

First-order difference equations

One of the simplest systems an ecologist can study is a seasonally breeding population in which generations do not overlap[1–4]. Many natural populations, particularly among temperate zone insects (including many economically important crop and orchard pests), are of this kind. In this situation, the observational data will usually consist of information about the maximum, or the average, or the total population in each generation. The theoretician seeks to understand how the magnitude of the population in generation $t+1$, X_{t+1}, is related to the magnitude of the population in the preceding generation t, X_t: such a relationship may be expressed in the general form

$$X_{t+1} = F(X_t) \qquad (1)$$

The function $F(X)$ will usually be what a biologist calls "density dependent", and a mathematician calls nonlinear; equation (1) is then a first-order, nonlinear difference equation.

Although I shall henceforth adopt the habit of referring to the variable X as "the population", there are countless situations outside population biology where the basic equation (1) applies. There are other examples in biology, as, for example in genetics[5,6] (where the equation describes the change in gene frequency in time) or in epidemiology[7] (with X the fraction of the population infected at time t). Examples in economics include models for the relationship between commodity quantity and price[8], for the theory of business cycles[9], and for the temporal sequences generated by various other economic quantities[10]. The general equation (1) also is germane to the social sciences[11], where it arises, for example, in theories of

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learning (where X may be the number of bits of information that can be remembered after an interval t), or in the propagation of rumours in variously structured societies (where X is the number of people to have heard the rumour after time t). The imaginative reader will be able to invent other contexts for equation (1).

In many of these contexts, and for biological populations in particular, there is a tendency for the variable X to increase from one generation to the next when it is small, and for it to decrease when it is large. That is, the nonlinear function $F(X)$ often has the following properties: $F(0)=0$; $F(X)$ increases monotonically as X increases through the range $0<X<A$ (with $F(X)$ attaining its maximum value at $X=A$); and $F(X)$ decreases monotonically as X increases beyond $X=A$. Moreover, $F(X)$ will usually contain one or more parameters which "tune" the severity of this nonlinear behaviour; parameters which tune the steepness of the hump in the $F(X)$ curve. These parameters will typically have some biological or economic or sociological significance.

A specific example is afforded by the equation[1,4,12–23]

$$N_{t+1} = N_t(a-bN_t) \tag{2}$$

This is sometimes called the "logistic" difference equation. In the limit $b=0$, it describes a population growing purely exponentially (for $a>1$); for $b\neq 0$, the quadratic nonlinearity produces a growth curve with a hump, the steepness of which is tuned by the parameter a. By writing $X=bN/a$, the equation may be brought into canonical form[1,4,12–23]

$$X_{t+1} = aX_t\,(1-X_t) \tag{3}$$

In this form, which is illustrated in Fig. 1, it is arguably the simplest nonlinear difference equation. I shall use equation (3) for most of the numerical examples and illustrations in this article. Although attractive to mathematicians by virtue of its extreme simplicity, in practical applications equation (3) has the disadvantage that it requires X to remain on the interval $0<X<1$; if X ever exceeds unity, subsequent iterations diverge towards $-\infty$ (which means the population becomes extinct). Furthermore, $F(X)$ in equation (3) attains a maximum value of $a/4$ (at $X=\frac{1}{2}$); the equation therefore possesses non-trivial dynamical behaviour only if $a<4$. On the other hand, all trajectories are attracted to $X=0$ if $a<1$. Thus for non-trivial dynamical behaviour we require $1<a<4$; failing this, the population becomes extinct.

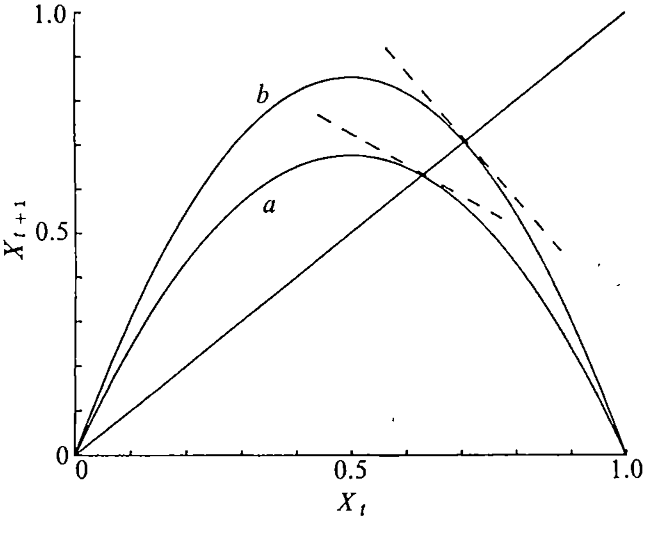


Fig. 1 A typical form for the relationship between X_{t+1} and X_t described by equation (1). The curves are for equation (3), with a = 2.707 (a); and a = 3.414 (b). The dashed lines indicate the slope at the "fixed points" where $F(X)$ intersects the 45° line: for the case a this slope is less steep than −45° and the fixed point is stable; for b the slope is steeper than −45°, and the point is unstable.

Another example, with a more secure provenance in the biological literature[1,23–27], is the equation

$$X_{t+1} = X_t\exp[r(1-X_t)] \tag{4}$$

This again describes a population with a propensity to simple exponential growth at low densities, and a tendency to decrease at high densities. The steepness of this nonlinear behaviour is tuned by the parameter r. The model is plausible for a single species population which is regulated by an epidemic disease at high density[28]. The function $F(X)$ of equation (4) is slightly more complicated than that of equation (3), but has the compensating advantage that local stability implies global stability[1] for all $X>0$.

The forms (3) and (4) by no means exhaust the list of single-humped functions $F(X)$ for equation (1) which can be culled from the ecological literature. A fairly full such catalogue is given, complete with references, by May and Oster[1]. Other similar mathematical functions are given by Metropolis *et al.*[16]. Yet other forms for $F(X)$ are discussed under the heading of "mathematical curiosities" below.

Dynamic properties of equation (1)

Possible constant, equilibrium values (or "fixed points") of X in equation (1) may be found algebraically by putting $X_{t+1}=X_t=X^*$, and solving the resulting equation

$$X^* = F(X^*) \tag{5}$$

An equivalent graphical method is to find the points where the curve $F(X)$ that maps X_t into X_{t+1} intersects the 45° line, $X_{t+1}=X_t$, which corresponds to the ideal nirvana of zero population growth; see Fig. 1. For the single-hump curves discussed above, and exemplified by equations (3) and (4), there are two such points: the trivial solution $X=0$, and a non-trivial solution X^* (which for equation (3) is $X^*=1-[1/a]$).

The next question concerns the stability of the equilibrium point X^*. This can be seen[24,25,19–21,1,4] to depend on the slope of the $F(X)$ curve at X^*. This slope, which is illustrated by the dashed lines in Fig. 1, can be designated

$$\lambda^{(1)}(X^*) = [\mathrm{d}F/\mathrm{d}X]_{X=X^*} \tag{6}$$

So long as this slope lies between 45° and −45° (that is, $\lambda^{(1)}$ between +1 and −1), making an acute angle with the 45° ZPG line, the equilibrium point X^* will be at least locally stable, attracting all trajectories in its neighbourhood. In equation (3), for example, this slope is $\lambda^{(1)}=2-a$: the equilibrium point is therefore stable, and attracts all trajectories originating in the interval $0<X<1$, if and only if $1<a<3$.

As the relevant parameters are tuned so that the curve $F(X)$ becomes more and more steeply humped, this stability-determining slope at X^* may eventually steepen beyond −45° (that is, $\lambda^{(1)}<-1$), whereupon the equilibrium point X^* is no longer stable.

What happens next? What happens, for example, for $a>3$ in equation (3)?

To answer this question, it is helpful to look at the map which relates the populations at successive intervals 2 generations apart; that is, to look at the function which relates X_{t+2} to X_t. This second iterate of equation (1) can be written

$$X_{t+2} = F[F(X_t)] \tag{7}$$

or, introducing an obvious piece of notation,

$$X_{t+2} = F^{(2)}(X_t) \tag{8}$$

The map so derived from equation (3) is illustrated in Figs 2 and 3.

Population values which recur every second generation (that

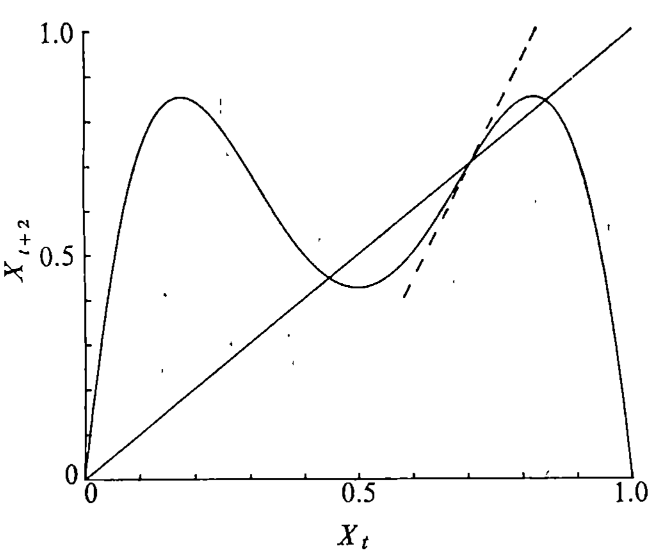


Fig. 2 The map relating X_{t+2} to X_t, obtained by two iterations of equation (3). This figure is for the case (*a*) of Fig. 1, $a = 2.707$: the basic fixed point is stable, and it is the only point at which $F^{(2)}(X)$ intersects the 45° line (where its slope, shown by the dashed line, is less steep than 45°).

is, fixed points with period 2) may now be written as X^*_2, and found either algebraically from

$$X^*_2 = F^{(2)}(X^*_2) \tag{9}$$

or graphically from the intersection between the map $F^{(2)}(X)$ and the 45° line, as shown in Figs 2 and 3. Clearly the equilibrium point X^* of equation (5) is a solution of equation (9); the basic fixed point of period 1 is a degenerate case of a period 2 solution. We now make a simple, but crucial, observation[1]: the slope of the curve $F^{(2)}(X)$ at the point X^*, defined as $\lambda^{(2)}(X^*)$ and illustrated by the dashed lines in Figs 2 and 3, is the square of the corresponding slope of $F(X)$

$$\lambda^{(2)}(X^*) = [\lambda^{(1)}(X^*)]^2 \tag{10}$$

This fact can now be used to make plain what happens when the fixed point X^* becomes unstable. If the slope of $F(X)$ is less than −45° (that is, $|\lambda^{(1)}| < 1$), as illustrated by curve *a* in Fig. 1, then X^* is stable. Also, from equation (10), this implies $0 < \lambda^{(2)} < 1$ corresponding to the slope of $F^{(2)}$ at X^* lying between 0° and 45°, as shown in Fig. 2. As long as the fixed point X^* is stable, it provides the only non-trivial solution to equation (9). On the other hand, when $\lambda^{(1)}$ steepens beyond −45° (that is, $|\lambda^{(1)}| > 1$), as illustrated by curve *b* in Fig 1, X^* becomes unstable. At the same time, from equation (10) this implies $\lambda^{(2)} > 1$, corresponding to the slope of $F^{(2)}$ at X^* steepening beyond 45°, as shown in Fig. 3. As this happens, the curve $F^{(2)}(X)$ must develop a "loop", and two new fixed points of period 2 appear, as illustrated in Fig. 3.

In short, as the nonlinear function $F(X)$ in equation (1) becomes more steeply humped, the basic fixed point X^* may become unstable. At exactly the stage when this occurs, there are born two new and initially stable fixed points of period 2, between which the system alternates in a stable cycle of period 2. The sort of graphical analysis indicated by Figs 1, 2 and 3, along with the equation (10), is all that is needed to establish this generic result[1,4].

As before, the stability of this period 2 cycle depends on the slope of the curve $F^{(2)}(X)$ at the 2 points. (This slope is easily shown to be the same at both points[1,20], and more generally to be the same at all *k* points on a period *k* cycle.) Furthermore, as is clear by imagining the intermediate stages between Figs 2 and 3, this stability-determining slope has the value $\lambda = +1$ at the birth of the 2-point cycle, and then decreases through zero towards $\lambda = -1$ as the hump in $F(X)$ continues to steepen. Beyond this point the period 2 points will in turn become unstable, and bifurcate to give an initially stable cycle of period 4. This in turn gives way to a cycle of period 8, and thence to a hierarchy of bifurcating stable cycles of periods 16, 32, 64, . . ., 2^n. In each case, the way in which a stable cycle of period *k* becomes unstable, simultaneously bifurcating to produce a new and initially stable cycle of period $2k$, is basically similar to the process just adumbrated for $k=1$. A more full and rigorous account of the material covered so far is in ref. 1.

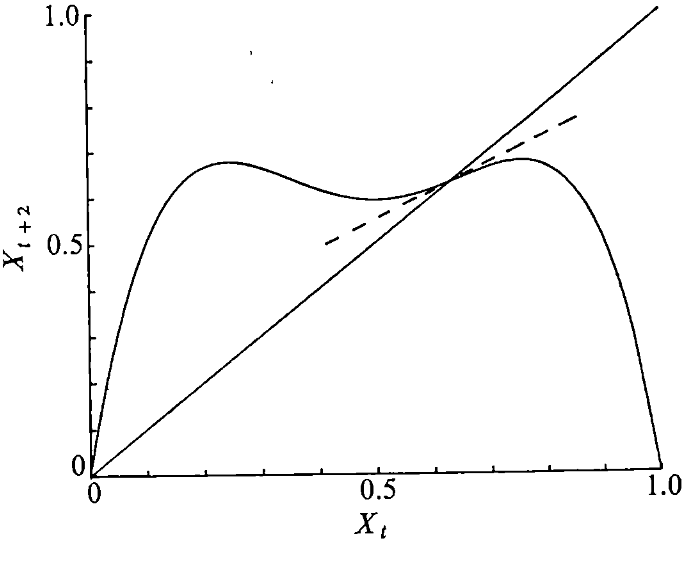
This "very beautiful bifurcation phenomenon"[22] is depicted in Fig. 4, for the example equation (3). It cannot be too strongly emphasised that the process is generic to most functions $F(X)$ with a hump of tunable steepness. Metropolis *et al.*[16] refer to this hierarchy of cycles of periods 2^n as the harmonics of the fixed point X^*.

Although this process produces an infinite sequence of cycles with periods 2^n $(n \rightarrow \infty)$, the "window" of parameter values wherein any one cycle is stable progressively diminishes, so that the entire process is a convergent one, being bounded above by some critical parameter value. (This is true for most, but not all, functions $F(X)$: see equation (17) below.) This critical parameter value is a point of accumulation of period 2^n cycles. For equation (3) it is denoted a_c: $a_c = 3.5700\ldots$

Beyond this point of accumulation (for example, for $a > a_c$ in equation (3)) there are an infinite number of fixed points with different periodicities, and an infinite number of different periodic cycles. There are also an uncountable number of initial points X_0 which give totally aperiodic (although bounded) trajectories; no matter how long the time series generated by $F(X)$ is run out, the pattern never repeats. These facts may be established by a variety of methods[1,4,20,22,29]. Such a situation, where an infinite number of different orbits can occur, has been christened "chaotic" by Li and Yorke[20].

As the parameter increases beyond the critical value, at first all these cycles have even periods, with X_t alternating up and down between values above, and values below, the fixed point X^*. Although these cycles may in fact be very complicated (having a non-degenerate period of, say, 5,726 points before repeating), they will seem to the casual observer to be rather like a somewhat "noisy" cycle of period 2. As the parameter value continues to increase, there comes a stage (at $a = 3.6786$ . . for equation (3)) at which the first odd period cycle appears. At first these odd cycles have very long periods, but as the parameter value continues to increase cycles with smaller and smaller odd periods are picked up, until at last the three-point

Fig. 3 As for Fig. 2, except that here $a = 3.414$, as in Fig. 1*b*. The basic fixed point is now unstable: the slope of $F^{(2)}(X)$ at this point steepens beyond 45°, leading to the appearance of two new solutions of period 2.



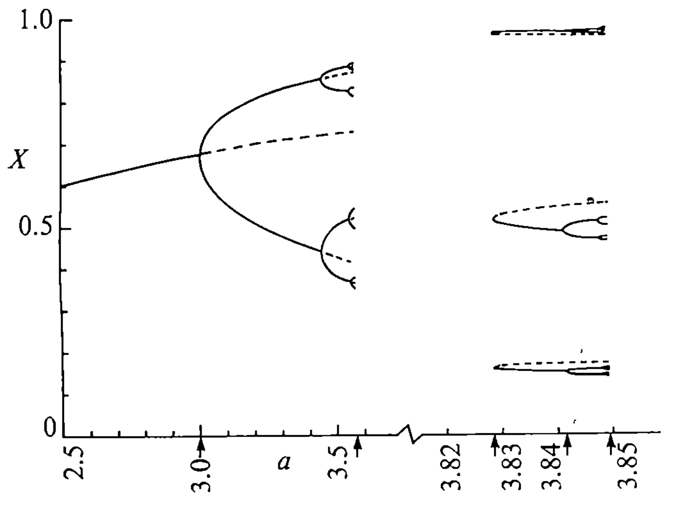


Fig. 4 This figure illustrates some of the stable (———) and unstable (— — — —) fixed points of various periods that can arise by bifurcation processes in equation (1) in general, and equation (3) in particular. To the left, the basic stable fixed point becomes unstable and gives rise by a succession of pitchfork bifurcations to stable harmonics of period 2^n; none of these cycles is stable beyond $a = 3.5700$. To the right, the two period 3 cycles appear by tangent bifurcation: one is initially unstable; the other is initially stable, but becomes unstable and gives way to stable harmonics of period 3×2^n, which have a point of accumulation at $a = 3.8495$. Note the change in scale on the a axis, needed to put both examples on the same figure. There are infinitely many other such windows, based on cycles of higher periods.

cycle appears (at $a = 3.8284$ . . for equation (3)). Beyond this point, there are cycles with every integer period, as well as an uncountable number of asymptotically aperiodic trajectories: Li and Yorke[20] entitle their original proof of this result "Period Three Implies Chaos".

The term "chaos" evokes an image of dynamical trajectories which are indistinguishable from some stochastic process. Numerical simulations[12,15,21,23,25] of the dynamics of equation (3), (4) and other similar equations tend to confirm this impression. But, for smooth and "sensible" functions $F(X)$ such as in equations (3) and (4), the underlying mathematical fact is that for any specified parameter value there is one unique cycle that is stable, and that attracts essentially all initial points[22,29] (see ref. 4, appendix A, for a simple and lucid exposition). That is, there is one cycle that "owns" almost all initial points; the remaining infinite number of other cycles, along with the asymptotically aperiodic trajectories, own a set of points which, although uncountable, have measure zero.

As is made clear by Tables 3 and 4 below, any one particular stable cycle is likely to occupy an extraordinarily narrow window of parameter values. This fact, coupled with the long time it is likely to take for transients associated with the initial conditions to damp out, means that in practice the unique cycle is unlikely to be unmasked, and that a stochastic description of the dynamics is likely to be appropriate, in spite of the underlying deterministic structure. This point is pursued further under the heading "practical applications", below.

The main messages of this section are summarised in Table 1, which sets out the various domains of dynamical behaviour of the equations (3) and (4) as functions of the parameters, a and r respectively, that determine the severity of the nonlinear response. These properties can be understood qualitatively in a graphical way, and are generic to any well behaved $F(X)$ in equation (1).

We now proceed to a more detailed discussion of the mathematical structure of the chaotic regime for analytical functions, and then to the practical problems alluded to above and to a consideration of the behavioural peculiarities exhibited by non-analytical functions (such as those in the two right hand columns of Table 1).

Fine structure of the chaotic regime

We have seen how the original fixed point X^* bifurcates to give harmonics of period 2^n. But how do new cycles of period k arise?

The general process is illustrated in Fig. 5, which shows how period 3 cycles originate. By an obvious extension of the notation introduced in equation (8), populations three generations apart are related by

$$X_{t+3} = F^{(3)}(X_t) \qquad (11)$$

If the hump in $F(X)$ is sufficiently steep, the threefold iteration will produce a function $F^{(3)}(X)$ with 4 humps, as shown in Fig. 5 for the $F(X)$ of equation (3). At first (for $a < 3.8284$ . . in equation 3) the 45° line intersects this curve only at the single point X* (and at $X=0$), as shown by the solid curve in Fig. 5. As the hump in $F(X)$ steepens, the hills and valleys in $F^{(3)}(X)$ become more pronounced, until simultaneously the first two valleys sink and the final hill rises to touch the 45° line, and then to intercept it at 6 new points, as shown by the dashed curve in Fig. 5. These 6 points divide into two distinct three-point cycles. As can be made plausible by imagining the intermediate stages in Fig. 5, it can be shown that the stability-determining slope of $F^{(3)}(X)$ at three of these points has a common value, which is $\lambda^{(3)} = +1$ at their birth, and thereafter steepens beyond $+1$: this period 3 cycle is never stable. The slope of $F^{(3)}(X)$ at the other three points begins at $\lambda^{(3)} = +1$, and then decreases towards zero, resulting in a stable cycle of period 3. As $F(X)$ continues to steepen, the slope $\lambda^{(3)}$ for this intially stable three-point cycle decreases beyond -1; the cycle becomes unstable, and gives rise by the bifurcation process discussed in the previous section to stable cycles of period 6, 12, 24, . . ., 3×2^n. This birth of a stable and unstable pair of period 3 cycles, and the subsequent harmonics which arise as the initially stable cycle becomes unstable, are illustrated to the right of Fig. 4.

Table 1 Summary of the way various "single-hump" functions $F(X)$, from equation (1), behave in the chaotic region, distinguishing the dynamical properties which are generic from those which are not

The function $F(X)$ of equation (1)	$aX(1-X)$	$X\exp[r(1-X)]$	aX; if $X<\frac{1}{2}$ $a(1-X)$; if $X>\frac{1}{2}$	λX; if $X<1$ λX^{1-b}; if $X>1$
Tunable parameter	a	r	a	b
Fixed point becomes unstable	3.0000	2.0000	1.0000*	2.0000
"Chaotic" region begins [point of accumulation of cycles of period 2^n]	3.5700	2.6924	1.0000	2.0000
First odd-period cycle appears	3.6786	2.8332	1.4142	2.6180
Cycle with period 3 appears [and therefore every integer period present]	3.8284	3.1024	1.6180	3.0000
"Chaotic" region ends	4.0000†	∞‡	2.000†	∞‡
Are there stable cycles in the chaotic region?	Yes	Yes	No	No

* Below this a value, $X = 0$ is stable.

† All solutions are attracted to $-\infty$ for a values beyond this.

‡ In practice, as r or b becomes large enough, X will eventually be carried so low as to be effectively zero, thus producing extinction in models of biological populations.

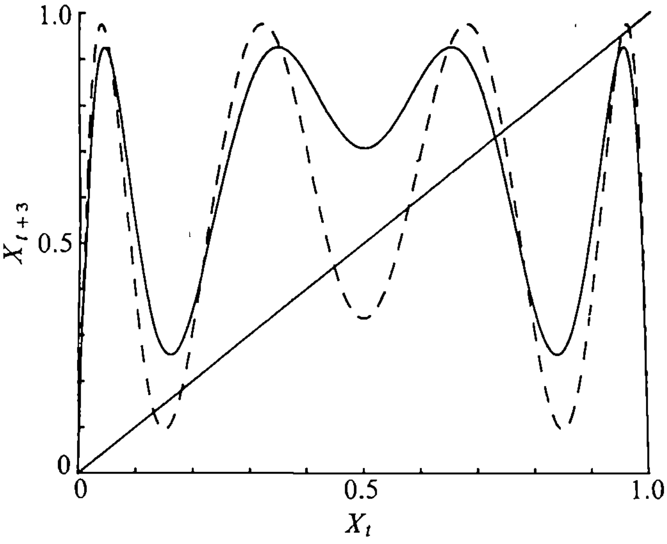
Table 2 Catalogue of the number of periodic points, and of the various cycles (with periods $k = 1$ up to 12), arising from equation (1) with a single-humped function $F(X)$

k	1	2	3	4	5	6	7	8	9	10	11	12
Possible total number of points with period k	2	4	8	16	32	64	128	256	512	1,024	2,048	4,096
Possible total number of points with non-degenerate period k	2	2	6	12	30	54	126	240	504	990	2,046	4,020
Total number of cycles of period k, including those which are degenerate and/or harmonics and/or never locally stable	2	3	4	6	8	14	20	36	60	108	188	352
Total number of non-degenerate cycles (including harmonics and unstable cycles)	2	1	2	3	6	9	18	30	56	99	186	335
Total number of non-degenerate, stable cycles (including harmonics)	1	1	1	2	3	5	9	16	28	51	93	170
Total number of non-degenerate, stable cycles whose basic period is k (that is, excluding harmonics)	1	–	1	1	3	4	9	14	28	48	93	165

There are, therefore, two basic kinds of bifurcation processes[1,4] for first order difference equations. Truly new cycles of period k arise in pairs (one stable, one unstable) as the hills and valleys of higher iterates of $F(X)$ move, respectively, up and down to intercept the 45° line, as typified by Fig. 5. Such cycles are born at the moment when the hills and valleys become tangent to the 45° line, and the initial slope of the curve $F^{(k)}$ at the points is thus $\lambda^{(k)}=+1$: this type of bifurcation may be called[1,4] a tangent bifurcation or a $\lambda=+1$ bifurcation. Conversely, an originally stable cycle of period k may become unstable as $F(X)$ steepens. This happens when the slope of $F^{(k)}$ at these period k points steepens beyond $\lambda^{(k)}=-1$, whereupon a new and initially stable cycle of period $2k$ is born in the way typified by Figs 2 and 3. This type of bifurcation may be called a pitchfork bifurcation (borrowing an image from the left hand side of Fig. 4) or a $\lambda=-1$ bifurcation[1,4].

Putting all this together, we conclude that as the parameters in $F(X)$ are varied the fundamental, stable dynamical units are cycles of basic period k, which arise by tangent bifurcation, along with their associated cascade of harmonics of periods $k2^n$, which arise by pitchfork bifurcation. On this basis, the constant equilibrium solution X^* and the subsequent hierarchy of stable cycles of periods 2^n is merely a special case, albeit a conspicuously important one (namely $k=1$), of a general phenomenon. In addition, remember[1,4,22,29] that for sensible, analytical functions (such as, for example, those in equations (3) and (4)) there is a unique stable cycle for each value of the parameter in $F(X)$. The entire range of parameter values ($1<a<4$ in equation (3), $0<r$ in equation (4)) may thus be regarded as made up of infinitely many windows of parameter values—some large, some unimaginably small—each corresponding to a single one of these basic dynamical units. Tables 3 and 4, below, illustrate this notion. These windows are divided from each other by points (the points of accumulation of the harmonics of period $k2^n$) at which the system is truly chaotic, with no attractive cycle: although there are infinitely many such special parameter values, they have measure zero on the interval of all values.

Fig. 5 The relationship between X_{t+3} and X_t, obtained by three iterations of equation (3). The solid curve is for $a = 3.7$, and only intersects the 45° line once. As a increases, the hills and valleys become more pronounced. The dashed curve is for $a = 3.9$, and six new period 3 points have appeared (arranged as two cycles, each of period 3)



How are these various cycles arranged along the interval of relevant parameter values? This question has to my knowledge been answered independently by at least 6 groups of people, who have seen the problem in the context of combinatorial theory[16,30], numerical analysis[13,14], population biology[1], and dynamical systems theory[22,31] (broadly defined).

A simple-minded approach (which has the advantage of requiring little technical apparatus, and the disadvantage of being rather clumsy) consists of first answering the question, how many period k points can there be? That is, how many distinct solutions can there be to the equation

$$X^*_k = F^{(k)}(X^*_k)? \qquad (12)$$

If the function $F(X)$ is sufficiently steeply humped, as it will be once the parameter values are sufficiently large, each successive iteration doubles the number of humps, so that $F^{(k)}(X)$ has 2^{k-1} humps. For large enough parameter values, all these hills and valleys will intersect the 45° line, producing 2^k fixed points of period k. These are listed for $k\leqslant 12$ in the top row of Table 2. Such a list includes degenerate points of period k, whose period is a submultiple of k; in particular, the two period 1 points ($X=0$ and X^*) are degenerate solutions of equation (12) for all k. By working from left to right across Table 2, these degenerate points can be subtracted out, to leave the total number of non-degenerate points of basic period k, as listed in the second row of Table 2. More sophisticated ways of arriving at this result are given elsewhere[13,14,16,22,30,31].

For example, there eventually are $2^6=64$ points with period 6. These include the two points of period 1, the period 2 "harmonic" cycle, and the stable and unstable pair of triplets of points with period 3, for a total of 10 points whose basic period is a submultiple of 6; this leaves 54 points whose basic period is 6.

The 2^k period k points are arranged into various cycles of period k, or submultiples thereof, which appear in succession by either tangent or pitchfork bifurcation as the parameters in $F(X)$ are varied. The third row in Table 2 catalogues the total number of distinct cycles of period k which so appear. In the fourth row[14], the degenerate cycles are subtracted out, to give the total number of non-degenerate cycles of period k: these numbers must equal those of the second row divided by k. This fourth row includes the (stable) harmonics which arise by pitchfork bifurcation, and the pairs of stable–unstable cycles arising by tangent bifurcation. By subtracting out the cycles which are unstable from birth, the total number of possible stable cycles is given in row five; these figures can also be obtained by less pedestrian methods[13,16,30]. Finally we may subtract out the stable cycles which arise by pitchfork bifurcation, as harmonics of some simpler cycle, to arrive at the final

Table 3 A catalogue of the stable cycles (with basic periods up to 6) for the equation $X_{t+1} = aX_t(1-X_t)$

Period of basic cycle	*a* value at which: Basic cycle first appears	*a* value at which: Basic cycle becomes unstable	Subsequent cascade of "harmonics" with period $k2^n$ all become unstable	Width of the range of *a* values over which the basic cycle, or one of its harmonics, is attractive
1	1.0000	3.0000	3.5700	2.5700
3	3.8284	3.8415	3.8495	0.0211
4	3.9601	3.9608	3.9612	0.0011
5(*a*)	3.7382	3.7411	3.7430	0.0048
5(*b*)	3.9056	3.9061	3 9065	0.0009
5(*c*)	3.99026	3.99030	3.99032	0.00006
6(*a*)	3.6265	3.6304	3.6327	0.0062
6(*b*)	3.937516	3.937596	3.937649	0.000133
6(*c*)	3.977760	3.977784	3.977800	0.000040
6(*d*)	3.997583	3.997585	3.997586	0.000003

row in Table 2, which lists the number of stable cycles whose basic period is k.

Returning to the example of period 6, we have already noted the five degenerate cycles whose periods are submultiples of 6. The remaining 54 points are parcelled out into one cycle of period 6 which arises as the harmonic of the only stable three-point cycle, and four distinct pairs of period 6 cycles (that is, four initially stable ones and four unstable ones) which arise by successive tangent bifurcations. Thus, reading from the foot of the column for period 6 in Table 2, we get the numbers 4, 5, 9, 14.

Using various labelling tricks, or techniques from combinatorial theory, it is also possible to give a generic list of the order in which the various cycles appear[1,13,16,22]. For example, the basic stable cycles of periods 3, 5, 6 (of which there are respectively 1, 3, 4) must appear in the order 6, 5, 3, 5, 6, 6, 5, 6: compare Tables 3 and 4. Metropolis *et al.*[16] give the explicit such generic list for all cycles of period $k \leqslant 11$.

As a corollary it follows that, given the most recent cycle to appear, it is possible (at least in principle) to catalogue all the cycles which have appeared up to this point. An especially elegant way of doing this is given by Smale and Williams[22], who show, for example, that when the stable cycle of period 3 first originates, the total number of other points with periods k, N_k, which have appeared by this stage satisfy the Fibonacci series, $N_k=2$, 4, 5, 8, 12, 19, 30, 48, 77, 124, 200, 323 for $k=1, 2, \ldots, 12$: this is to be contrasted with the total number of points of period k which will eventually appear (the top row of Table 2) as $F(X)$ continues to steepen.

Such catalogues of the total number of fixed points, and of their order of appearance, are relatively easy to construct. For any particular function $F(X)$, the numerical task of finding the windows of parameter values wherein any one cycle or its harmonics is stable is, in contrast, relatively tedious and inelegant. Before giving such results, two critical parameter values of special significance should be mentioned.

Hoppensteadt and Hyman[21] have given a simple graphical method for locating the parameter value in the chaotic regime at which the first odd period cycle appears. Their analytic recipe is as follows. Let α be the parameter which tunes the steepness of $F(X)$ (for example, $\alpha=a$ for equation (3), $\alpha=r$ for equation (4)), $X^*(\alpha)$ be the fixed point of period 1 (the nontrivial solution of equation (5)), and $X_{max}(\alpha)$ the maximum value attainable from iterations of equation (1) (that is, the value of $F(X)$ at its hump or stationary point). The first odd period cycle appears for that value of α which satisfies[21,31]

$$X^*(\alpha) = F^{(2)}(X_{max}(\alpha)) \qquad (13)$$

As mentioned above, another critical value is that where the period 3 cycle first appears. This parameter value may be found numerically from the solutions of the third iterate of equation (1): for equation (3) it is[14] $a=1+\sqrt{8}$.

Myrberg[13] (for all $k \leqslant 10$) and Metropolis *et al.*[16]. (for all $k \leqslant 7$) have given numerical information about the stable cycles in equation (3). They do not give the windows of parameter values, but only the single value at which a given cycle is maximally stable; that is, the value of a for which the stability-determining slope of $F^{(k)}(X)$ is zero, $\lambda^{(k)}=0$. Since the slope of the k-times iterated map $F^{(k)}$ at any point on a period k cycle is simply equal to the product of the slopes of $F(X)$ at each of the points X^*_k on this cycle[1,8,20], the requirement $\lambda^{(k)}=0$ implies that $X=A$ (the stationary point of $F(X)$, where $\lambda^{(1)}=0$) is one of the periodic points in question, which considerably simplifies the numerical calculations.

For each basic cycle of period k (as catalogued in the last row of Table 2), it is more interesting to know the parameter values at which: (1) the cycle first appears (by tangent bifurcation); (2) the basic cycle becomes unstable (giving rise by successive pitchfork bifurcations to a cascade of harmonics of periods $k2^n$); (3) all the harmonics become unstable (the point of accumulation of the period $k2^n$ cycles). Tables 3 and 4 extend the work of May and Oster[1], to give this numerical information for equations (3) and (4), respectively. (The points of accumulation are not ground out mindlessly, but are calculated by a rapidly convergent iterative procedure, see ref. 1, appendix A.) Some of these results have also been obtained by Gumowski and Mira[32].

Practical problems

Referring to the paradigmatic example of equation (3), we can now see that the parameter interval $1<a<4$ is made up of a one-dimensional mosaic of infinitely many windows of a-values, in each of which a unique cycle of period k, or one of its harmonics, attracts essentially all initial points. Of these windows, that for $1<a<3.5700$ . . corresponding to $k=1$ and its harmonics is by far the widest and most conspicuous. Beyond the first point of accumulation, it can be seen from Table 3 that these windows are narrow, even for cycles of quite low periods, and the windows rapidly become very tiny as k increases.

As a result, there develops a dichotomy between the underlying mathematical behaviour (which is exactly determinable) and the "commonsense" conclusions that one would draw from numerical simulations. If the parameter a is held constant at one value in the chaotic region, and equation (3) iterated for an arbitrarily large number of generations, a density plot of the observed values of X_t on the interval 0 to 1 will settle into k equal spikes (more precisely, delta functions) corresponding to the k points on the stable cycle appropriate to this a-value. But for most a-values this cycle will have a fairly large period, and moreover it will typically take many thousands of generations before the transients associated with the initial conditions are damped out: thus the density plot produced by numerical simulations usually looks like a sample of points taken from some continuous distribution.

An especially interesting set of numerical computations are due to Hoppensteadt (personal communication) who has combined many iterations to produce a density plot of X_t for each one of a sequence of a-values, gradually increasing from 3.5700 . . to 4. These results are displayed as a movie. As can be expected from Table 3, some of the more conspicuous cycles do show up as sets of delta functions: the 3-cycle and its first few harmonics; the first 5-cycle; the first 6-cycle. But for most values of a the density plot looks like the sample function of a random process. This is particularly true in the neighbourhood of the a-value where the first odd cycle appears ($a=3.6786\,.\,.$), and again in the neighbourhood of $a=4$: this is not surprising, because each of these locations is a point of accumulation of points of accumulation. Despite the underlying discontinuous changes in the periodicities of the stable cycles, the observed density pattern tends to vary smoothly. For example, as a increases toward the value at which the 3-cycle appears, the density plot tends to concentrate around three points, and it smoothly diffuses away from these three points after the 3-cycle and all its harmonics become unstable.

I think the most interesting mathematical problem lies in designing a way to construct some approximate and "effectively continuous" density spectrum, despite the fact that the exact density function is determinable and is always a set of delta functions. Perhaps such techniques have already been developed in ergodic theory[33] (which lies at the foundations of statistical mechanics), as for example in the use of "coarse-grained observers". I do not know.

Such an effectively stochastic description of the dynamical properties of equation (4) for large r has been provided[28], albeit by tactical tricks peculiar to that equation rather than by any general method. As r increases beyond about 3, the trajectories generated by this equation are, to an increasingly good approximation, almost periodic with period $(1/r)\exp(r-1)$.

The opinion I am airing in this section is that although the exquisite fine structure of the chaotic regime is mathematically fascinating, it is irrelevant for most practical purposes. What seems called for is some effectively stochastic description of the deterministic dynamics. Whereas the various statements about the different cycles and their order of appearance can be made in generic fashion, such stochastic description of the actual dynamics will be quite different for different $F(X)$: witness the difference between the behaviour of equation (4), which for large r is almost periodic "outbreaks" spaced many generations apart, versus the behaviour of equation (3), which for $a\rightarrow 4$ is not very different from a series of Bernoulli coin flips.

Mathematical curiosities

As discussed above, the essential reason for the existence of a succession of stable cycles throughout the "chaotic" regime is that as each new pair of cycles is born by tangent bifurcation (see Fig. 5), one of them is at first stable, by virtue of the way the smoothly rounded hills and valleys intercept the 45° line. For analytical functions $F(X)$, the only parameter values for which the density plot or "invariant measure" is continuous and truly ergodic are at the points of accumulation of harmonics, which divide one stable cycle from the next. Such exceptional parameter values have found applications, for example, in the use of equation (3) with $a=4$ as a random number generator[34,35]: it has a continuous density function proportional to $[X(1-X)]^{-\frac{1}{2}}$ in the interval $0<X<1$.

Non-analytical functions $F(X)$ in which the hump is in fact a spike provide an interesting special case. Here we may imagine spikey hills and valleys moving to intercept the 45° line in Fig. 5, and it may be that both the cycles born by tangent bifurcation are unstable from the outset (one having $\lambda^{(k)}>1$, the other $\lambda^{(k)}<-1$), for all $k>1$. There are then no stable cycles in the chaotic regime, which is therefore literally chaotic with a continuous and truly ergodic density distribution function.

One simple example is provided by

$$\begin{aligned} X_{t+1} &= aX_t;\ \text{if}\ X_t<\tfrac{1}{2} \\ X_{t+1} &= a(1-X_t);\ \text{if}\ X_t>\tfrac{1}{2} \end{aligned} \qquad (14)$$

defined on the interval $0<X<1$. For $0<a<1$, all trajectories are attracted to $X=0$; for $1<a<2$, there are infinitely many periodic orbits, along with an uncountable number of aperiodic trajectories, none of which are locally stable. The first odd period cycle appears at $a=\surd 2$, and all integer periods are represented beyond $a=(1+\surd 5)/2$. Kac[36] has given a careful discussion of the case $a=2$. Another example, this time with an extensive biological pedigree[1-3], is the equation

$$\begin{aligned} X_{t+1} &= \lambda X_t;\ \text{if}\ X_t<1 \\ X_{t+1} &= \lambda X_t^{1-b};\ \text{if}\ X_t>1 \end{aligned} \qquad (15)$$

If $\lambda>1$ this possesses a globally stable equilibrium point for $b<2$. For $b>2$ there is again true chaos, with no stable cycles: the first odd cycle appears at $b=(3+\surd 5)/2$, and all integer periods are present beyond $b=3$. The dynamical properties of equations (14) and (15) are summarised to the right of Table 2.

The absence of analyticity is a necessary, but not a sufficient, condition for truly random behaviour[31]. Consider, for example,

$$\begin{aligned} X_{t+1} &= (a/2)X_t;\ \text{if}\ X_t<\tfrac{1}{2} \\ X_{t+1} &= aX_t(1-X_t)\ ;\ \text{if}\ X_t>\tfrac{1}{2} \end{aligned} \qquad (16)$$

This is the parabola of equation (3) and Fig. 1, but with the left hand half of $F(X)$ flattened into a straight line. This equation does possess windows of a values, each with its own stable cycle, as described generically above. The stability-determining slopes $\lambda^{(k)}$ vary, however, discontinuously with the parameter a, and the widths of the simpler stable regions are narrower than for equation (3): the fixed point becomes unstable at $a=3$; the point of accumulation of the subsequent harmonics is at $a=3.27\,.\,.$; the first odd cycle appears at $a=3.44\,.\,.$; the 3-point cycle at $a=3.67\,.\,.$ (compare the first column in Table 1).

These eccentricities of behaviour manifested by non-analytical functions may be of interest for exploring formal questions in ergodic theory. I think, however, that they have no relevance to models in the biological and social sciences, where functions such as $F(X)$ should be analytical. This view is elaborated elsewhere[37].

As a final curiosity, consider the equation

$$X_{t+1} = \lambda X_t[1+X_t]^{-\beta} \qquad (17)$$

Table 4 Catalogue of the stable cycles (with basic periods up to 6) for the equation $X_{t+1} = X_t\exp[r(1-X_t)]$

Period of basic cycle	r value at which: Basic cycle first appears	Basic cycle becomes unstable	Subsequent cascade of "harmonics" with period $k2^n$ *all* become unstable	Width of the range of r values over with the basic cycle, or one of its harmonics, is attractive
1	0.0000	2.0000	2.6924	2.6924
3	3.1024	3.1596	3.1957	0.0933
4	3.5855	3.6043	3.6153	0.0298
5(*a*)	2.9161	2.9222	2.9256	0.0095
5(*b*)	3.3632	3.3664	3.3682	0.0050
5(*c*)	3.9206	3.9295	3.9347	0.0141
6(*a*)	2.7714	2.7761	2.7789	0.0075
6(*b*)	3.4558	3.4563	3.4567	0.0009
6(*c*)	3.7736	3.7745	3.7750	0.0014
6(*d*)	4.1797	4.1848	4.1880	0.0083

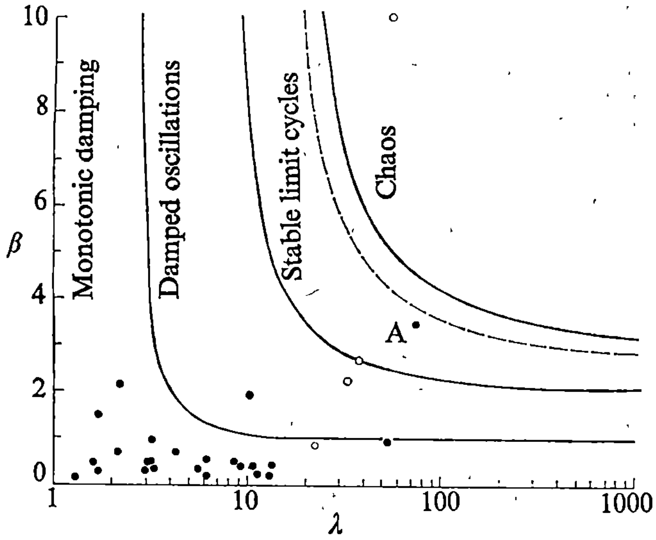


Fig. 6 The solid lines demarcate the stability domains for the density dependence parameter, β, and the population growth rate, λ, in equation (17); the dashed line shows where 2-point cycles give way to higher cycles of period 2^n. The solid circles come from analyses of life table data on field populations, and the open circles from laboratory populations (from ref. 3, after ref. 39).

This has been used to fit a considerable amount of data on insect populations[38,39]. Its stability behaviour, as a function of the two parameters λ and β, is illustrated in Fig. 6. Notice that for $\lambda < 7.39$ . . there is a globally stable equilibrium point for all β; for $7.39 .. < \lambda < 12.50 ..$ this fixed point becomes unstable for sufficiently large β, bifurcating to a stable 2-point cycle which is the solution for all larger β; as λ increases through the range $12.50 .. < \lambda < 14.77 ..$ various other harmonics of period 2^n appear in turn. The hierarchy of bifurcating cycles of period 2^n is thus truncated, and the point of accumulation and subsequent regime of chaos is not achieved (even for arbitrarily large β) until $\lambda > 14.77$ . . .

Applications

The fact that the simple and deterministic equation (1) can possess dynamical trajectories which look like some sort of random noise has disturbing practical implications. It means, for example, that apparently erratic fluctuations in the census data for an animal population need not necessarily betoken either the vagaries of an unpredictable environment or sampling errors: they may simply derive from a rigidly deterministic population growth relationship such as equation (1). This point is discussed more fully and carefully elsewhere[1].

Alternatively, it may be observed that in the chaotic regime arbitrarily close initial conditions can lead to trajectories which, after a sufficiently long time, diverge widely. This means that, even if we have a simple model in which all the parameters are determined exactly, long term prediction is nevertheless impossible. In a meteorological context, Lorenz[16] has called this general phenomenon the "butterfly effect": even if the atmosphere could be described by a deterministic model in which all parameters were known, the fluttering of a butterfly's wings could alter the initial conditions, and thus (in the chaotic regime) alter the long term prediction.

Fluid turbulence provides a classic example where, as a parameter (the Reynolds number) is tuned in a set of deterministic equations (the Navier–Stokes equations), the motion can undergo an abrupt transition from some stable configuration (for example, laminar flow) into an apparently stochastic, chaotic regime. Various models, based on the Navier–Stokes differential equations, have been proposed as mathematical metaphors for this process[15,40,41]. In a recent review of the theory of turbulence, Martin[42] has observed that the one-dimensional difference equation (1) may be useful in this context. Compared with the earlier models[15,40,41], it has the disadvantage of being even more abstractly metaphorical, and the advantage of having a spectrum of dynamical behaviour which is more richly complicated yet more amenable to analytical investigation.

A more down-to-earth application is possible in the use of equation (1) to fit data[1,2,3,38,39,43] on biological populations with discrete, non-overlapping generations, as is the case for many temperate zone arthropods. Figure 6 shows the parameter values λ and β that are estimated[39] for 24 natural populations and 4 laboratory populations when equation (17) is fitted to the available data. The figure also shows the theoretical stability domains: a stable point; its stable harmonics (stable cycles of period 2^n); chaos. The natural populations tend to have stable equilibrium point behaviour. The laboratory populations tend to show oscillatory or chaotic behaviour; their behaviour may be exaggeratedly nonlinear because of the absence, in a laboratory setting, of many natural mortality factors. It is perhaps suggestive that the most oscillatory natural population (labelled *A* in Fig. 6) is the Colorado potato beetle, whose present relationship with its host plant lacks an evolutionary pedigree. These remarks are only tentative, and must be treated with caution for several reasons. Two of the main caveats are that there are technical difficulties in selecting and reducing the data, and that there are no single species populations in the natural world: to obtain a one-dimensional difference equation by replacing a population's interactions with its biological and physical environment by passive parameters (such as λ and β) may do great violence to the reality.

Some of the many other areas where these ideas have found applications were alluded to in the second section, above[5–11]. One aim of this review article is to provoke applications in yet other fields.

Related phenomena in higher dimensions

Pairs of coupled, first-order difference equations (equivalent to a single second-order equation) have been investigated in several contexts[4,44–46], particularly in the study of temperate zone arthropod prey–predator systems[2–4,23,47]. In these two-dimensional systems, the complications in the dynamical behaviour are further compounded by such facts as: (1) even for analytical functions, there can be truly chaotic behaviour (as for equations (14) and (15)), corresponding to so-called "strange attractors"; and (2) two or more different stable states (for example, a stable point and a stable cycle of period 3) can occur together for the same parameter values[4]. In addition, the manifestation of these phenomena usually requires less severe nonlinearities (less steeply humped $F(X)$) than for the one-dimensional case.

Similar systems of first-order ordinary differential equations, or two coupled first-order differential equations, have much simpler dynamical behaviour, made up of stable and unstable points and limit cycles[48]. This is basically because in continuous two-dimensional systems the inside and outside of closed curves can be distinguished; dynamic trajectories cannot cross each other. The situation becomes qualitatively more complicated, and in many ways analogous to first-order difference equations, when one moves to systems of three or more coupled, first-order ordinary differential equations (that is, three-dimensional systems of ordinary differential equations). Scanlon (personal communication) has argued that chaotic behaviour and "strange attractors", that is solutions which are neither points nor periodic orbits[48], are typical of such systems. Some well studied examples arise in models for reaction–diffusion systems in chemistry and biology[49], and in the models of Lorenz[15] (three dimensions) and Ruelle and Takens[40] (four dimensions) referred to above. The analysis of these systems is, by virtue of their higher dimensionality, much less transparent than for equation (1).

An explicit and rather surprising example of a system which has recently been studied from this viewpoint is the ordinary differential equations used in ecology to describe competing species. For one or two species these systems are very tame: dynamic trajectories will converge on some stable equilibrium point (which may represent coexistence, or one or both species becoming extinct). As Smale[50] has recently shown, however, for 3 or more species these general equations can, in a certain reasonable and well-defined sense, be compatible with any dynamical behaviour. Smale's[50] discussion is generic and abstract: a specific study of the very peculiar dynamics which can be exhibited by the familiar Lotka-Volterra equations once there are 3 competitors is given by May and Leonard[51].

Conclusion

In spite of the practical problems which remain to be solved, the ideas developed in this review have obvious applications in many areas.

The most important applications, however, may be pedagogical.

The elegant body of mathematical theory pertaining to linear systems (Fourier analysis, orthogonal functions, and so on), and its successful application to many fundamentally linear problems in the physical sciences, tends to dominate even moderately advanced University courses in mathematics and theoretical physics. The mathematical intuition so developed ill equips the student to confront the bizarre behaviour exhibited by the simplest of discrete nonlinear systems, such as equation (3). Yet such nonlinear systems are surely the rule, not the exception, outside the physical sciences.

I would therefore urge that people be introduced to, say, equation (3) early in their mathematical education. This equation can be studied phenomenologically by iterating it on a calculator, or even by hand. Its study does not involve as much conceptual sophistication as does elementary calculus. Such study would greatly enrich the student's intuition about nonlinear systems.

Not only in research, but also in the everyday world of politics and economics, we would all be better off if more people realised that simple nonlinear systems do not necessarily possess simple dynamical properties.

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articles

Prenatal genesis of connections subserving ocular dominance in the rhesus monkey

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In foetal monkey brain neuronal projections carrying input from the two eyes initially overlap; they segregate during the second half of gestation and become fully separated in subcortical visual centres and partially separated in the cortex three weeks before birth and thus before visual experience.

DEVELOPMENT of the binocular visual system has been a major topic of research since the demonstration more than a decade ago that neuronal function can be altered in kittens deprived of vision in one eye[1]. More recently, similar observations in the rhesus monkey[2] have underlined the relevance of this research to an understanding of mechanisms of amblyopia *ex* anopsia in children. In several respects, the development of the visual system is similar in

Table 1 Experimental protocol for three foetal monkeys injected at various times for autoradiography

Animal	Embryonic day (E) when injected	Embryonic day (E) when killed	Exposure time for LGd and SCsg (weeks)	Exposure time for cortex (weeks)
(1) 052775	E64	E78	2, 4	2, 4, 8, 16, 20, 24
(2) 021075	E110	E124	2, 4	2, 4, 8, 16, 20, 24
(3) 061275	E130	E144	2, 4	2, 4, 8, 16, 20, 24

Three foetal monkeys were injected once each at the specified embryonic (E) day into one eye with equal parts of ^{3}H-proline and ^{3}H-fucose After injection, each foetus was replaced in the uterus and killed 14 d later.

man and monkey. For example, in both species all neurones that comprise the visual system are generated during the first two-thirds of gestation[3–5]. In both species, approximately one-half of the axons from the retinal ganglion cells of each eye are distributed at the optic chiasma to both the ipsilateral and the contralateral dorsal lateral geniculate bodies (LGd). In both man and monkey, the LGd has 6 laminae. Three laminae (1, 4 and 6) receive direct inputs from the contralateral eye and the remaining three (2, 3 and 5) from the ipsilateral eye[6].

The recently developed autoradiographic method of orthograde axoplasmic[7] and transneuronal transport[8,9] provide a simple and powerful method for exploring the structural development of the binocular visual system by tracing the distribution of radioactive label following injection of isotopes into one eye. Radioactivity transported to the appropriate laminae of the LGd and transneuronally to the primary visual cortex (area 17 of Brodmann) is distributed in the sublayers IVA and IVC over columns 350 μm wide that alternate with columns of the same width containing low grain counts[9]. The autoradiographic method thus confirms earlier more traditional anatomical and electrophysiological evidence for columnar organisation. It has also provided the first anatomical evidence for a segregated representation of each eye in the superior colliculus (SC), where fibres from the two eyes terminate in complementary, alternating territories 0.1–0.5 mm wide[10,11].

Autoradiography has also been used to determine the anatomical consequences of monocular deprivation at early postnatal ages. This method reveals a reciprocal expansion and contraction of the ocular dominance columns in layer IV of the visual cortex that are related to non-occluded and occluded eyes, respectively[12]. It has been suggested that the basic connections subserving binocular vision are determined innately, even though, as mentioned above, changes in the width of columns can be induced by an imbalance in visual experience during the first few postnatal months[12]. This interpretation, however, has not been fully accepted by some investigators who argue that visual experience rather than genetic factors may play a fundamental inductive role in establishing functional connections. (For a recent review on the subject, see ref. 13.) An analysis of the prenatal development of the circuitry involved in ocular dominance in the monkey may therefore help to resolve the issue.

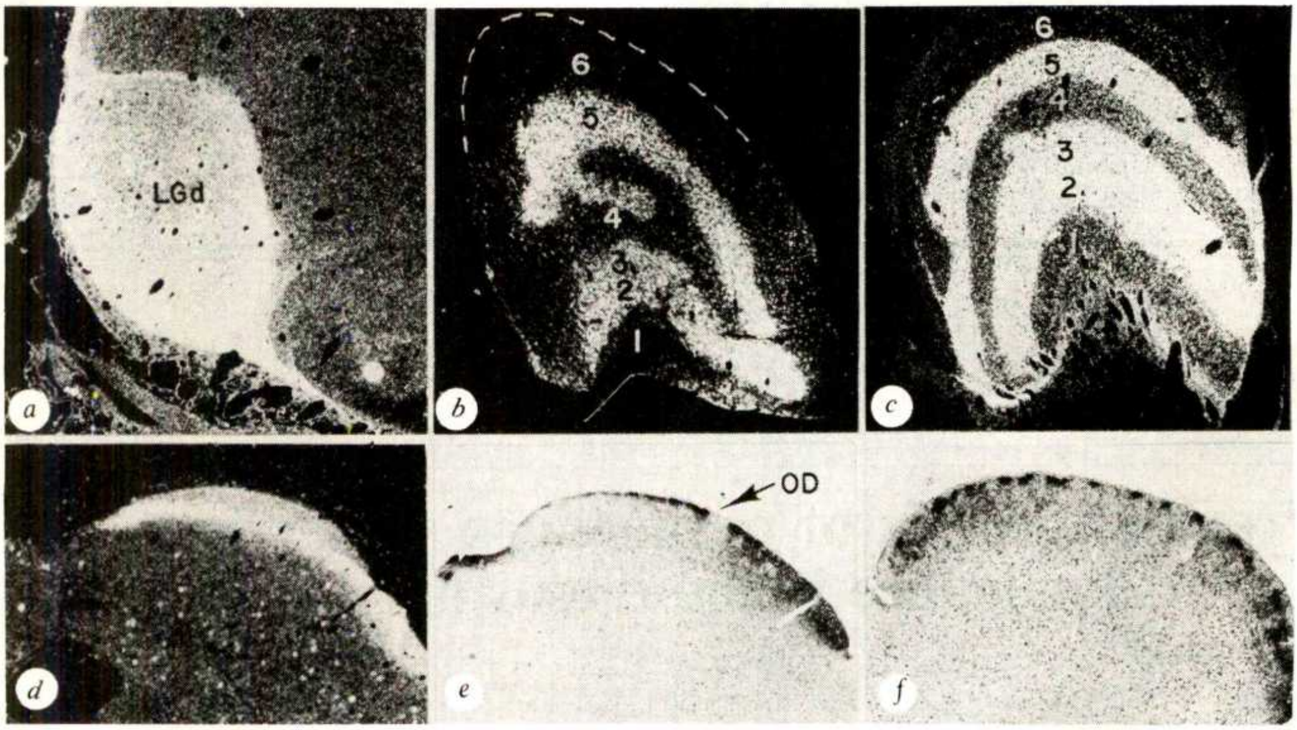


Fig. 1 Dark-field and bright-field illumination photographs of autoradiograms from foetuses exposed by hysterotomy and injected into the vitreous body of one eye with a mixture of ^{3}H-proline and ^{3}H-fucose at different embryonic (E) days. Foetuses were replaced in the uterus and killed 14 d later by a second caesarian section. All photographs are the same magnification (×20). The terms "contralateral" and "ipsilateral" are used with respect to the injected eye. *a*, Contralateral LGd in the foetus injected at E64 and killed at E78. The radioactive label does not suggest a laminar pattern. *b*, Ipsilateral LGd in the foetus injected with comparable doses of the same isotopes at E110, killed at E124. Label is concentrated over the emerging laminae 2, 3 and 5. *d*, Dark-field photograph of a coronal section of the ipsilateral superior colliculus (SC) of the foetus injected at E64 and killed at E78. Label is uniformly distributed over the entire nucleus. *e*, Bright-field photograph of a sagittal section of the contralateral SC in the foetus injected at E110 and killed at E124. Retinotectal projections are clearly segregated into ocular dominance patches indicated by alternating areas of high and low grain counts. The large, empty zone corresponds to the optic disk (OD) representation. *f*, Bright-field photograph of a coronal section of the ipsilateral SC in the foetus injected and killed at slightly later gestational ages (E130–E144). Retinotectal projections are concentrated into discrete patches of dense labelling that correspond to areas devoid of grains in the contralateral SC. The optic disk representation is not present at this level.

Pregnant rhesus monkeys (*Maccaca mulatta*) were subjected to laparotomy and hysterotomy under halothane–oxygen anaesthesia at appropriate gestational intervals (Table 1). Equal amounts of ^{3}H-proline and ^{3}H-fucose (total 1.0–1.5 mCi) were injected into the vitreous body of one eye of each foetus. After injection, the foetuses were returned to the uterus, and the chorio-allantoic membrane, uterine and abdominal walls were sutured in layers. Fourteen days later, after an interval sufficient for concentration of tracer in LGd, SC and visual cortex by axonal and transneuronal transport, the living foetuses were removed from the uterus by a second caesarian section and perfused intracardially with a buffered paraformaldehyde–glutaraldehyde mixture. The brains were embedded in polyester wax, sectioned serially at 8 μm and processed by the standard autoradiographic method.

Retinogeniculate projections

The youngest foetus in this series was injected at E64 (embryonic day 64) by which time all LGd neurones have been generated[14]. This foetus was killed at E78, before the LGd becomes laminated[5]. Thus, this experiment was designed to determine whether retinal ganglion cell axons from each eye are segregated in the LGd before it develops laminae.

Radioactive tracer, transported in an orthograde direction from ganglion cells in the injected eye to the optic nerve, through the optic chiasma and the optic tracts, is concentrated in approximately equal amounts in both LGds. Evidently, equal numbers of crossed and uncrossed retinal fibres are already distributed to each LGd at this foetal age. Although there are areas with slightly higher or lower grain densities, segregation into separate layers is not discernible (Fig. 1*a*). Rather, grains are distributed more or less diffusely throughout the entire ipsilateral and contralateral LGd, indicating the possibility that at an early stage of development (around E64) projections from both eyes basically overlap in their distribution among neurones of the LGd with no evidence of the laminar segregation characteristically seen in the adult nucleus.

The second foetus in this series was injected in one eye with a mixture of ^{3}H-proline and ^{3}H-fucose at E110, that is, at an age when the characteristic laminar pattern of the LGd is beginning to emerge[5]. Fourteen days later, at E124, when this foetus was killed, the six-layered pattern of cells of the LGd, although still somewhat irregular, is already clearly developed. The laminae 2, 3 and 5 of the LGd are found to be intensely labelled ipsilateral to the injected eye (Fig. 1*b*) whereas laminae 1, 4 and 6 are labelled in the contralateral nucleus. The laminar segregation of ipsilateral and contralateral retinal inputs to LGd therefore occurs in the interval between E64 and E110 when laminar segregation of their target cells is also achieved. In both LGd, the more ventral laminae (one contralaterally and two ipsilaterally) are more intensely labelled than the more dorsal ones.

The distribution of radioactive label in a third foetus injected and killed at slightly later gestational ages (E130–E144) is essentially the same, except that the distribution of grains over the appropriate laminae resembles closely the adult patern (Fig. 1*c*); that is ,the laminar organisation of cells in the LGd is more regular and more sharply outlined than seen in younger foetuses.

Retinotectal projections

Substantial amounts of radioactivity were transported through the brachium of the superior colliculus into the tectum in all three foetuses. In the youngest foetus, which was injected at E64 and killed at E78, the tracer is concentrated uniformly over the superficial layer of both SC with no evidence of complementary segregation of projections from the ipsilateral and contralateral eyes (Fig. 1*d*). The amount of label is noticeably greater on the contralateral side, however. Fibres from the two eyes therefore initially overlap in the SC, as well as in the LGd.

By contrast, in the foetus injected at E110 and killed at E124, the radioactive label is distributed in a pattern of alternating dense and light concentrations (Fig. 1*e*). Although not as sharply defined, these correspond closely in number and pattern to the ocular dominance 'clumps' observed in the mature monkey[11]. The alternating pattern is most evident in the posterior region of the SC where the retinal axons penetrate more deeply into the superficial grey. The width of the 'clumps' in the foetal monkey, ranging from 0.07 to 0.3 mm, is substantially smaller than that of the adult monkey (0.1–0.5 mm)[11], presumably reflecting the smaller size of the SC in the foetus. As in the adult, the pattern of grain distribution on both sides fits like pieces in a puzzle; that is, territories of the contralateral SC which contain low concentrations correspond to territories of the ipsilateral SC which contain high concentrations. In addition, a sector which contains no label in the contralateral SC represents the position of the optic disk at this foetal age (OD in Fig. 1*e*).

The oldest foetus in this study, injected at E130 and killed at E144, shows essentially the same pattern of alternating grain distribution in the SC. The contrast between heavily and lightly labelled territories is more distinct and the boundaries of these territories are defined as sharply as in the adult monkey (Fig. 1*f*).

Geniculocortical projections

Radioactive label was transported transneuronally into neurones of the LGd and the geniculocortical fibres of the three foetuses as revealed by a high concentration of label of the optic radiations (OR in Fig. 2*a*). Because the amount of radioactivity transferred transneuronally is less than 3% of that transported through the primary pathway[8], autoradiographic exposures were lengthened appropriately for demonstration of geniculocortical projections (Table 1). As expected, this was done at the expense of relatively low background (Fig. 2).

The youngest foetus was injected at E64 and killed at E78, that is when only a fraction of neurones destined for layer IV of the visual cortex are generated[3], and many of these neurones which have already undergone their last cell division have not yet reached their final position and are still migrating towards the cortex through the fibre plexus of the intermediate zone[4]. The autoradiograms show that fibres from the optic radiation (OR in Fig. 2*a*) have reached the occipital lobe, but they have not entered the developing cortex in any substantial number, and most of the label is concentrated in the subcortical intermediate zone of the developing cerebral wall. There is an abrupt decline in grain density at the external border of this zone (SP in Fig. 2*a*) which is situated external to the optic radiation but internal to the developing cortex. The distribution of grains in the intermediate zone forms a continuous, uniform band without periodic variations. Thus, at this stage in the telencephalon, there is no evidence of segregation of fibres derived from the two eyes. This is not unexpected since, in the same foetus, axons of the retinal projections from the two eyes are not yet segregated in the LGd (see above).

The second foetus was injected at E110 and was killed at E124, that is, after all cortical neurones are generated and also after they have completed their migration to the cortex[4]. In this foetus, the location of LGd axons are clearly identified by a dense concentration of grains over the optic radiation (OR) in the occipital lobe (Fig. 2*b*). Numerous fibres pass from the optic radiation towards the cortex of area 17, but stop abruptly at the border of this field with area 18 (Fig. 2*b*). Although the great majority of these fibres is concentrated in the intermediate zone

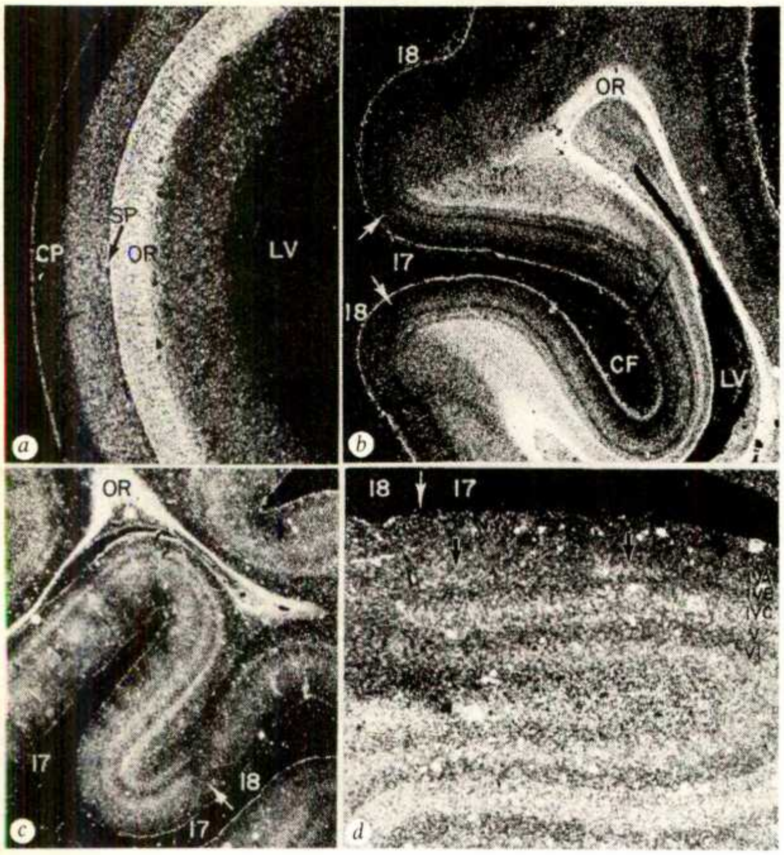


Fig. 2 Dark-field photographs of the cerebral wall and cortex at the level of the occipital lobe in the foetus injected with a mixture of ^{3}H-proline and ^{3}H-fucose at various foetal ages and killed 14 d later. The high grain background in these autoradiograms is due in part to the long exposure (Table 1) and partly to the high dose of ^{3}H-proline–^{3}H-fucose mixture that produces unspecific labelling similar to background levels found in autoradiograms of liver, kidney, spleen and other organs prepared from the same foetuses. Therefore, only a concentration of grains well above background in the autoradiograms of the cerebrum is considered to represent radioactivity in the axons of the visual pathways. *a*, A coronal cut across the cerebral wall in the foetus injected at E64 and killed at E78. The large lateral vesicle (LV) is on the right. The optic radiation (OR) is heavily labelled, but fibres stop at the subcortical zone (SP) and do not invade the developing cortical plate (CP). Approximately ×27. *b*, Occipital lobe of the foetus whose one eye was injected with the same mixture of labels at E110 and killed at E124. The optic radiation (OR) surrounds the lateral ventricle (LV) and emanates axons only to area 17 of the calcarine fissure (CF). Projections to the cortex stop sharply at the borderline between area 17 and area 18 (arrows). Some axons and/or terminals have entered the cortical plate but are distributed uniformly over layers IV and VI. Approximately ×8.7. *c*, Visual cortex of the foetus injected and killed at a slightly older age (E130–E144). More axons and/or terminals have invaded the cortex and are concentrated over layers IV and VI in area 17. ×12. *d*, Higher magnification photograph of the visual cortex demonstrates segregation of axons and/or terminals over sublayers IVA and IVC, as well as alternating regions of higher (black arrowheads) and lower grain counts. The limits of zones with high grain densities in both layers occur sharply at the borderline to area 18. ×30.

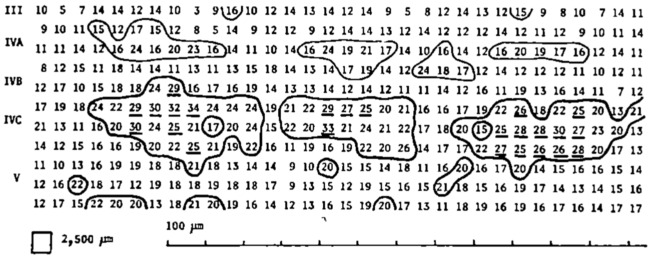
below the primary visual area, small numbers of them do enter the cortex, where they are uniformly distributed over layers IV and VI (Fig. 2*b*). There is, however, no horizontal bilaminar concentration of silver grains over sublayers IVA and IVC and no alternating radially aligned concentrations of grains that might indicate the incipient formation of ocular dominance columns.

In the third foetus that was injected and killed at a slightly later age (E130–E144) more grains are concentrated over layers IV and VI presumably because more geniculocortical fibres have invaded the cortex. In this specimen, a bilaminate pattern of grain concentration can be discerned over sublayers IVA and IVC in autoradiograms exposed 12 to 24 weeks (Fig. 2*c*). The boundaries between these two sublayers are not as sharp as in the adult, however. By and large, the grains over sublayers IVA and IVC at this foetal age are distributed as a continuous sheet, although there are sectors in which an alternating light and dense pattern of grain concentration seems to emerge (Fig. 2*c*). This pattern is of low contrast and, although difficult to see in photomicrographs, it is clearly present when a grain count is performed (Fig. 3). Sectors with higher counts in sublayers IVA are in register with those of IVC, and the same is true of sectors with lower counts (Figs 2*c* and 3). In all likelihood, these represent the emerging ocular dominance columns. Presumably, only the sectors, 100–150 μm wide, which contain the highest grain counts (underlined numerals in Fig. 3) and those with the lowest grain counts (numerals between outlines) correspond to territories occupied predominantly by terminals derived from the injected and uninjected eye, respectively. Therefore, the width of the sectors with high concentration of grains is not 50% of the double columns (Fig. 3) which indicates that there is still considerable overlap between the terminals derived from the two eyes. The combined width of an ipsilateral and contralateral ocular dominance column as determined by the distance between two peaks in grain concentrations is 500–600 μm. The comparable figure for the width of two columns in the mature monkey is 700–800 μm (ref. 9). The difference in width may be explained by a substantial increase in the cortical surface area between E144 and maturity.

Sequence of developmental events

The topographical interrelationship of the central connections in the adult visual system is the result of multiple, complex cellular interactions and events which occur during ontogeny. During the extended prenatal period of primates, young neurones change their position relative to one another; many of them migrate long distances to attain their final locations; and their dendritic and axonal processes differentiate and are deployed while the entire brain changes its external and internal form dramatically[4,5,14]. These cellular activities are coordinated in space and in time and may play a fundamental role in the formation of complex cytoarchitectonic patterns and point-to-point connectivity of widely separated visual centres.

These observations indicate that cellular events fundamental to the prenatal development of the visual system in the rhesus monkey may be separated into two broad phases: in the first phase, axons derived from each eye invade target structures, and their endings are distributed in an overlapping manner; in the second phase, the terminal axons derived from the two eyes become segregated from each other in their target structures. Although injections of isotopes at closer intervals and shorter survival times are needed to define the duration of each phase, the available evidence suggests that the first phase may be relatively short, even in the protracted development of the rhesus monkey, whereas the second phase may last for several weeks. Thus, in the foetus injected at E110 and killed at E124, afferents from the LGd are distributed in an overlapping manner over the entire IVth layer of the visual cortex. Even 6 weeks later, on the seventh postnatal day, the segregation into monocular columns is far from



III	10	5	7	14	14	12	14	10	3	9	16	10	12	14	13	14	12	14	9	5	8	12	14	13	12	15	9	8	10	7	14	11
IVA	9	10	11	15	12	17	15	12	8	5	14	9	12	12	9	12	14	14	13	12	14	12	12	12	14	12	11	12	9	10	11	14
	11	11	14	12	16	24	16	20	23	16	14	11	10	14	16	24	19	21	17	14	10	16	14	12	16	20	19	17	16	12	14	11
IVB	8	12	15	11	18	14	14	11	13	11	13	15	18	14	13	14	17	19	14	12	24	18	17	12	14	12	12	12	11	10	12	11
	12	17	10	15	18	18	24	29	16	17	16	19	14	13	13	14	12	14	12	11	11	14	12	16	11	19	13	16	14	11	7	12
IVC	17	19	18	24	22	29	30	32	34	24	24	24	19	21	22	29	27	25	20	21	16	16	17	19	22	26	18	22	25	20	13	21
	21	13	11	16	20	30	24	25	21	17	20	24	15	22	20	33	21	24	21	22	17	18	20	15	25	28	28	30	27	23	20	13
	14	12	15	16	16	19	20	22	25	21	19	22	16	11	19	16	19	22	20	26	14	17	17	22	27	25	26	26	28	20	17	13
V	11	10	13	16	19	19	18	18	21	18	13	14	14	9	10	20	15	15	14	18	11	16	20	16	17	20	14	15	16	16	15	14
	12	16	22	18	17	12	19	18	18	19	18	18	17	9	13	15	12	19	15	16	15	21	18	15	16	19	17	14	13	14	15	16
	12	17	15	22	20	20	13	18	21	20	19	16	14	12	13	16	15	19	20	17	13	11	18	19	16	19	16	17	16	14	17	17

Fig. 3 Results of grain counts over a 0 8-mm^2 area of layers III, IVA, IVB, IVC and V of the primary visual cortex in monkey foetuses injected at E130 and killed at E144 The autoradiogram is photographed in dark-field illumination and its negative projected by a Prado (Leitz GMBH) apparatus on to grid paper in which each square corresponds to 2,500 μm^2 area of the cortex Grains were counted in 352 unit areas along a 1 6-mm length of the calcarine cortex Areas with 15 grains or more per 2,500 μm^2 in layer IVA are outlined by a thin line Areas with 20 grains or more per 2,500 μm^2 in layer IVC are outlined by a thick line Areas with 25 grains or more are underscored by a short, thick line. The distance between the two regions of highest grain counts that correspond to the pair of ocular dominance columns is approximately 500 μm Note that the width of areas with higher grain counts is not half of the double column Presumably, territories of terminals from the uninjected eye still considerably overlap the territories of injected eye and only a narrow, approximately 100–150-μm wide zone of highest and lowest grain counts may be considered to correspond predominantly to areas which receive projections derived predominantly from one eye

complete[12]. It is important to emphasise that although differentiation of pathways begins later in the cortex than in subcortical structures, the initial segregation of binocular connections in the cortex nevertheless begins well before birth—that is, before visual experience

Thus the effect of monocular deprivation on the relative size of territories corresponding to each eye[12] may not necessarily involve the induced expansion of territories related to the functional eye Rather, since domains of ocular representation overlap with each other during ontogeny (Fig. 3), it is possible that the territory of the functional eye remains arrested at a given developmental stage, while the territories of the deprived eye shrink. In this context, our developmental data support the hypothesis that alterations in the relative width of ocular dominance columns could be degradative rather than inductive.

Recently, autoradiographic studies have demonstrated that radial columnar organisation is not confined to the primary visual cortex but is a generalised characteristic of the mammalian neocortex[15,16]. For example, in the motor and prefrontal cortex of the neonatal monkey, callosal and cortico-cortical connections form well developed, sharply delineated columns whose development at this age is much more advanced than that of the ocular dominance columns of the visual cortex[16]. The precocious development of columns in the frontal lobe of the monkey adds further support to the notion that columnar organisation of the cortex may be largely specified innately. The significant alteration of monocular dominance columns by unbalanced visual stimulation during early stages of postnatal life in primates may occur because columns are not yet fully developed.

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Mayan chronology and 'the spectrum of time'

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By applying methods used in varve[1] and tree-ring[2] analysis to link the floating Mayan chronology with the anchored chronology of calculated planetary positions a new correlation has been derived. The intervals between certain Mayan dates are known to be multiples of the synodic periods of several planets. Mayan astronomers are assumed to have observed close conjunctions of two or more planets, this is tested by comparisons with observed or calculated conjunctions. I conclude that the Mayan dates as conventionally expressed are 27.3 yr too old The suggested correlation number is 594,250±1 d, the difference between the Julian and the Mayan day numbers.

DATES inscribed on numerous Mayan monuments in Central America during the first millenium AD can readily be converted to days in a floating time scale, but the correct dates in the Christian calendar have hitherto been uncertain Many of the dates are now known to refer to the lives of the local rulers, but a few are 'astronomical'. Those dates of astronomical significance have been investigated in a computer study by Kelley and Kerr[3], who, by knowing the mean planetary periods, have determined which planets are associated with certain of the dates.

Any Mayan date, such as 9.16.4.10.8. (the base of the Dresden Codex 'Eclipse' table), can thus be converted to the number of days elapsed since the starting point of Mayan chronology this date, 9.16 4 10.8., is thus equivalent to a Mayan day number (MDN) of 1,412,848. Christian dates can similarly be converted to Julian day numbers (JDN), and to solve the Mayan Correlation Problem we must determine for the Classic period the correct constant difference between any linked pair of these day numbers.

Other chronologies

The conventional equation is that of Goodman–Martinez–Thompson which makes the date 9.10.0.0.0. (MDN 1,368,000) equivalent to January 22, AD 633 (JDN 1,952,283); the Thompson correlation is thus 584,283 and this is usually regarded as correct by the sixteenth century, when Spanish interpreters attempted to explain the Aztec calender.

Astronomers have not been satisfied with this equation and each specialist now seems to have a separate solution. In the recent AAS symposium[4] at Mexico at least six were discussed, those of 487,410 (Owen), 482,699 (Smiley) and 584,283 (Hatch) being separately adopted in the published papers. The radiocarbon dates, combined with archaeological opinion, suggest that the correlation of 584,283 is not more than 30,000 d in error, and the late Sir Eric Thompson admitted "Any correlation, deduced on other grounds, that makes 9.10.0.0.0 coincide with a conjunction of Venus and Jupiter would have much to recommend it." (see ref. 3, p.197). These two limiting conditions suggested the present investigation.

Data for new correlation

Observations of planetary conjunctions from China and elsewhere have been received from Professor E. H. Schafer and other contributors to the 'Spectrum of Time' project[5], and the positions checked with planetary positions calculated by Tuckerman[6]. All possible comparisons between the Mayan dates and the observations in the T'ang Histories were made by the writer for a range of sixty years either side of the conventional date, and one solution only (594,250 ± 35) provided a consistent correlation for several conjunctions. The Venus–Jupiter conjunction of 650 was one recorded by the Chinese; this conjunction was later regarded by Masha'allah[7] as signifying the fall of the Sasanians and the rise of the Arabs. This correlation dates the Venus–Jupiter conjunction of AD 650 near the Mayan date (9 10.0.0.0) required.

Another conjunction now brought into alignment with a Mayan date in this way is that of 660, one not specifically recorded in the Old World This conjunction, with a similar one two years later, appears to explain a letter of the Syrian Bishop Sebokht written about 662 Sebokht had been asked by the priest Basil from Cyprus whether there was any possibility of a conjunction of all the planets. John Philoponus in his polemic against Proclus had earlier mentioned a conjunction of 7 planets (that is including the Sun and Moon) in Taurus "in our time in the year 245 of Diocletian" (AD 529) and Sebokht in his reply confirmed this conjunction using contemporary tables of planetary positions. Calculations show that in 660 Jupiter and Venus were in the same longitude at about June 3 and likewise Jupiter and Mercury during the last half of the month.

Evidence from Caracol

Table 1 lists the dates in the inscription of Caracol (stela 3) selected by Kelley and Kerr[3] for their summary chart; also indicated are the names of planets selected by the computer analysis and assumed to be those observed and recorded on this ephemeris.

The calculated[6] longitudinal conjunctions near the dates cited are shown for comparison. Planetary conjunctions are not necessarily single events, some recurring within a few months or years so that discrepancies of up to 30 d are reasonable, but Mayan dates seem to have been adjusted slightly for some unknown reason. The stela can be interpreted in the light of the correlation[8]. In 653 Kelley's chart includes the date in his Saturn but not his Mercury column; although Jupiter was in conjunction with Mercury but not Saturn, Kelley's expectations in including the date in both his Saturn and Venus tables are confirmed by a Saturn–Venus conjunction on October 4 at a longitude separated by 45° from the other conjunction.

The Dresden Codex

The two dates in the Dresden Codex when most planets (according to the calculated periods of ref. 3) were involved can now be aligned on the new time scale as follows:

8.16.14.11.5. = 1,272,465 (MDN) = 1,866,715 (JDN)
= October 15, 398
9.12.10.16.9. = 1,386,329 (MDN) = 1,980,579 (JDN)
= July 13, 710

The former date is included in every planetary column except that for Jupiter (ref. 3, p. 210–211), and indeed it is found from Tuckerman that in November and December in that year all planets except Jupiter were in the same longitudes. A similar situation occurred in late June 710, Jupiter this time being involved as well; this is no doubt why calculations were made by I-Hsing at about AD 710, according to Needham[9], concerning the date of the last general conjunction. By multiplying the planetary periods together I-Hsing made it 96,961,740 yr ago, a Chinese calculation nearly as extravagant as corresponding calculations by the Mayans. There was also a visible eclipse of the Sun in 710 on May 3 and this is possibly referred to in the still undeciphered text.

Many other conjunctions can be dated in the monuments. In the Dresden Codex the earliest is that of June 8, 293 which is the exact date of a Jupiter–Mercury conjunction; the conjunctions of this year were observed by the Chinese[10] who reported merely that "Saturn, Jupiter and Venus assembled at the 19th and 18th lunar mansions".

Table 1 Some dates from the inscription of stela 3 at Caracol

Mayan date	Planets involved	MDN (+594,250)	Date		Approximate dates of conjunction (after Tuckerman[6])		
9.6.12.4.16.	Saturn–Venus	1,937,866	August 3,	593	July,	593	Saturn–Venus–Mercury
9.7.14.10.8.	Jupiter–Venus	1,945,898	July 31,	615	August 27,	615	Jupiter–Venus
9 9.10.0.0.	Saturn–Jupiter–Mercury	1,958,650	June 29,	650	June 9,	650	Saturn–Jupiter–Mercury
					June 12,	650	Saturn–Jupiter
9.9.13.4.4.	Saturn–Jupiter–Venus	1,959,814	September 4,	653	Early September,	653	Mercury–Jupiter
					August 16,	653	Saturn–Sun
					October 4,	653	Saturn–Venus
9.9.14.3.5.	Saturn–Mercury	1,960,155	August 12,	654	July 30,	654	Saturn–Mercury
9.9.18.16.3.	Jupiter–Venus	1,961,853	April 6,	659	March 13,	659	Jupiter–Venus
9.10.0.0.0.	Saturn–Jupiter–Venus	1,962,250	May 9,	660	June 3,	660	Jupiter–Venus
					October 24,	660	Saturn–Venus

The latest Long Count date in this Codex is that of AD 1238 according to the 594,250 correlation suggested here. This is the date (9.16.4.10 8.) already mentioned as the base of the Eclipse table (ref. 3, p. 189). This does not coincide with an eclipse in the Goodman—Martinez–Thompson correlation, but on the new correlation there was a lunar eclipse visible to the Mayans before the following sunrise (January 2). The Dresden dates will be discussed separately, but, on this new basis, the Eclipse Table would have provided the Maya with predictions of every important lunar eclipse visible to them (±2 d) for over 30 yr; the table also predicted almost every eclipse visible in other parts of the world, and it seems probable that the illustrations represented an unsuccessful attempt to differentiate between the two classes. The new correlation likewise makes possible a new interpretation of other dates associated with glyphs of the Sun, the Moon, Venus and eclipses.

Conclusion

Cross correlation of calculated conjunctions with the observed Mayan dates confirmed that there was no alternative solution within 250 yr using the known cycles of 60 and 24 yr in Saturn–Jupiter and Venus–Jupiter conjunctions. The Goodman–Martinez–Thompson solution therefore needs an addition of 27.3 yr, corresponding to my suggested correlation of 594,250 d.

The various dates examined by Kelley, and other dates in the Dresden Codex and elsewhere, have been tabulated[11], and longitude–time charts have been drawn for the planets, the Sun and the Moon. Computer comparison with the associated glyphs should thus make it possible to identify the glyphs for the remaining planets, and, using the known dates of comets[12] in the Old World, to identify the glyph for comet. The writer would be very grateful for any collaboration in this project.

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Geochemistry and diagenesis of deep-sea sediments from leg 35 of the Deep Sea Drilling Project

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The principal cause of ^{18}O depletion, Ca^{2+} increases, and Mg^{2+} decreases in the pore fluids of cores from leg 35 of the Deep Sea Drilling Project (DSDP) is the alteration of basalt, volcanic ash, and igneous components of terrigenous material. Post-depositional diagenetic reactions have produced smectites, zeolites, K-feldspar, and celadonite as the main authigenic products. $^{18}O/^{16}O$ data from calcite and clay minerals suggest that these reactions took place over a range of times, temperatures (10–60 °C), and pore fluid $^{18}O/^{16}O$ compositions. At site 323, the sediments 30–74 m above basement are enriched in Mn, Fe, Cu, Ni, Co, Mo and Sr. Below these horizons, Ba shows a sharp increase towards basement. The $^{13}C/^{12}C$ values ($\delta = -18.4$ to $-21.2‰$ PDB) of authigenic calcites in the sediments reflect an organic matter source for the carbon.

LAYER IIA (extrusive volcanics) and layer I (sediments) of the oceanic crust undergo major chemical and mineralogical transformations from the time of their extrusion or deposition to the time of their subduction. These transformations cause chemical changes in the pore fluids which, in turn, produce chemical changes in the oceans. The directions of chemical exchanges are known, but the locations and the sizes of the major chemical sinks and sources remain poorly defined[1–3].

Diagenetic reactions are recorded by chemical, mineralogical, and isotopic changes in the sediments, in their pore fluids, and in the underlying basalts. Decreases in dissolved sulphate concentration, along with increases in dissolved ammonia and alkalinity, are principally a reflection of sulphate reduction by bacteria[4,5]. Increases in calcium and decreases in magnesium, potassium, and $^{18}O/^{16}O$, however, are caused by mineralogical transformations in the sediments and basalts. Increases of calcium with depth are not always caused by dissolution and/or recrystallisation of $CaCO_3$ (refs 5 and 6) but can originate from

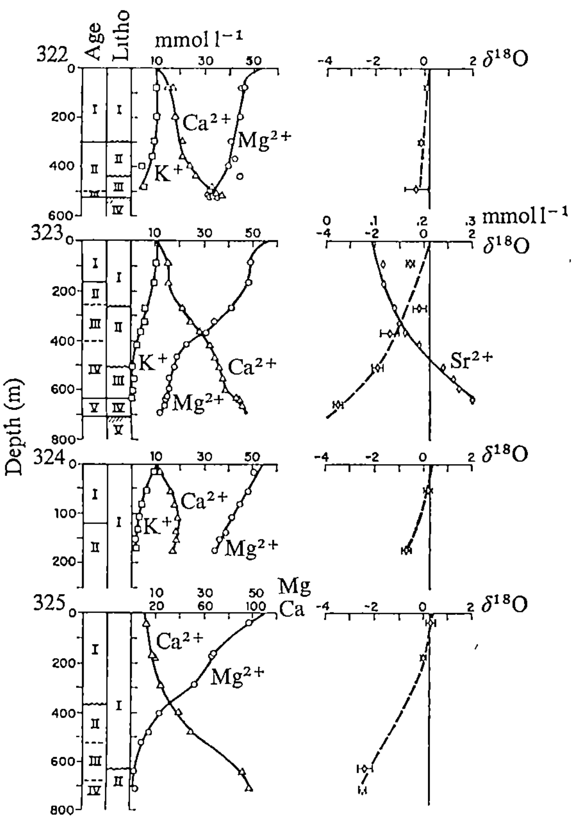

Fig. 1 Ca^{2+}, Mg^{2+}, K^+ and $\delta^{18}O$ gradients in interstitial fluids of leg 35, sites 322, 323, 324 and 325 DSDP.

the alteration of calcium-containing igneous phases. With the exception of sediments overlying evaporites, and some rapidly depositing sediments, a decrease in magnesium almost always accompanies an increase in calcium. Silicate transformations that involve magnesium exchange for calcium, as well as potassium and sodium uptake, are the most probable cause for the observed pore water gradients.

It was long thought that the amount of solids undergoing alteration should be small and, therefore, difficult to detect. With the discovery of $^{18}O/^{16}O$ decreases in the pore fluids that accompany major cation gradients, however, it became apparent that in many instances the amount of solids involved in the reactions had to be large[7], and it has been shown that the observed $^{18}O/^{16}O$ and major cation gradients are caused mainly by the alteration of volcanic ash or basalt[8-10].

Here we present the highlights of an intensive collaborative effort to delineate more clearly the major sinks and sources for the elements mentioned above. The focus is on the elemental and stable isotope chemistry of the interstitial waters and the chemistry and mineralogy of the sediments and basalts, leg 35 of DSDP in the Bellingshausen abyssal plain area of the southern Pacific Ocean.

Details of the sites

The changes in Ca^{2+}, Mg^{2+}, K^+ and $\delta^{18}O$ of the pore fluids are shown in Fig. 1, and the age and lithology of leg 35 sites in Table 1. All four sites show increases in Ca^{2+} and decreases in Mg^{2+}, K^+, and $\delta^{18}O$ with depth, but each site has its own signature. Clearly, the number and location of reaction zones are not the same in each site. Average slopes and changes in slopes of the concentration gradients are variable, being functions of extent and type of reactions, reaction rates and sedimentation rates, as well as the physical properties of the sediments. Dissolved Mg^{2+} reaches a concentration of almost zero only in site 325, which has the largest average sedimentation rate. A marked contrast in the amount of $\delta^{18}O$ depletion exists between sites 322 and 323. Numerous and most extensive diagenetic changes were observed in site 323.

Site 323

Studies of the diffusional transport properties of the sediments[11] have led to the conclusion that the dissolved strontium gradient (Fig 1) > 639 m arises essentially from diffusion from a 'point' source, probably located in the Sr-enriched sediments just below this boundary (Fig. 2). By comparing the Ca^{2+}, Mg^{2+}, and K^+ gradients in the pore fluids with the Sr^{2+} concentration depth profile, changes in slope can be interpreted with confidence in terms of reaction zones. Accordingly, three principal zones can be recognised:

a, the rapidly deposited Pliocene section (75–100 m); *b*, the zone of silification (400–510 m); and *c*, the basal sediments, consisting mostly of authigenic smectite and some clinoptilolite (640–700 m) and/or the underlying partially weathered basalts.

In zone *a*, weathering of plagioclase and/or mafic minerals is probably the source for Ca^{2+}, and authigenic clays are the most likely sinks for Mg^{2+} and K^+. In zone *b*, at ~ 450 m, Kastner[12] demonstrated from SEM photographs and X-ray energy dispersive chemical measurements that weathering of plagioclase and dissolution of nannoplankton are the sources of Ca^{2+} and authigenic smectite and K-feldspar are the sinks for Mg^{2+} and K^+.

An abrupt change in mineralogy of the sediments takes place at 639 m in zone (*c*), immediately below a large sedimentation hiatus of ~ 35×10^6 yr (ref. 13) Above, in the clay-size fraction, illite and mixed layered illite-smectite of 60–70% expandable layers are the most abundant clay minerals. This is also reflected in the relatively high K/Al ratios in the clay fraction (Fig. 2). Below, the dominant clay mineral is smectite and the K/Al values are low in the clay-size fractions. Of interest, however, is the observation that the K/Al ratio of the bulk sediment is nearly the same above and below this boundary[14]. Evidently the lower sediment has its K_2O preferentially located in relatively coarse phases (K-feldspar and clinoptilolite)[13].

Interpretation

The above mineralogical and chemical differences leave little doubt about the diagenetic nature of the sediments below

Table 1 Details of the sites

Site 322—60°01.45'S, 79°25.49'W; 5,026 m		
Age	I:	Pleistocene/Pliocene
	II:	Miocene
	III:	Oligocene?
Lithology	I:	sand; silt; clay
	II:	claystone
	III:	sandstone and claystone
	IV:	basalt
Site 323—63°40.84'S, 97°59 69'W; 4,993 m		
Age	I:	Pleistocene/Pliocene
	II:	Late Miocene
	III:	Middle Miocene
	IV:	Early Miocene
	V:	Palaeocene and older
Lithology	I:	clay; silt; sand
	II:	diatomite; claystone; chert < 400 m
	III:	claystone
	IV:	zeolitic clay
	V:	basalt
Site 324—69°03.21'S, 98°47 20'W; 4,449 m		
Age	I:	Pleistocene
	II:	Pliocene
Lithology	I:	clay; silt; claystone
Site 325—65°02 79'S, 73°40.40'W; 3,745 m		
Age	I:	Pleistocene/Pliocene
	II:	Late Miocene
	III:	Middle Miocene
	IV:	Early Miocene
Lithology	I:	clay; silt; claystone
	II:	claystone and silicified claystones

the hiatus. Changes in the mineralogy of the clays suggest a volcanic source[13]. Glass and palagonite have been reported in these sediments[12]. Donnelly and Wallace[14], however, emphasise the essentially isochemical nature of the bulk sediments above and below the hiatus, particularly with regard to the Ti/Al ratio (0.031) and the contents of K_2O, Na_2O, and MgO. Exceptions, of course, are the contrasting values of Fe, Mn, and P. On the basis of these data, they suggest that the sediments < 639 m are mainly of a terrigenous origin but have undergone substantial alteration to form the above-mentioned suite of minerals.

Pore water concentration gradients in Ca^{2+}, Mg^{2+} and K^+ in zone *c* cannot be interpreted with certainty as involving reactions in these sediments but may be related to ongoing alteration of underlying basalts, which show the presence of alteration products[12,13]. Typically, $\delta^{13}C$ values in calcite in the uppermost basalts are as low as −3‰ (PDB)[15], probably implying biogenic degradation of organic matter. The pyroxenes of the basalts are more extensively weathered than the plagioclases. Both are likely to provide Ca^{2+} to the pore fluids. Smectites in basalt veins may constitute important sinks for Mg^{2+}. The K^+ depletion observed < 639 m may be related to uptake in authigenic zeolites in the basal sediments and/or authigenic K-feldspars.

The large sedimentation hiatus previously mentioned implies either a long period of non-deposition or rather slow sedimentation rates and subsequent erosion by bottom currents during the onset of the circumpolar current. In either case, part or all of the alteration observed below the hiatus or in the basal sediments may have occurred before the Miocene, that is, before the renewed buildup of sediments. Observed pore water gradients < 500 m may then be caused principally by continued alteration of the underlying basalts.

Between 639 and 670 m, 30–74 m above the basement, the solids showed an enrichment in transition elements and strontium[13,14,18]. Below this, barium shows a sharp increase towards basement (Fig. 2). We interpret this in terms of hydrothermal events in the underlying basalts which have led to an upward advection of these hydrothermal fluids with the subsequent precipitation of barite near the basalt–sediment interface and the formation of transition metal oxides in the oxygenated upper sediments. The observations of the occurrence of celadonite[12] and saponite[13] in the basalts, and of well crystallised clay minerals in the basal sediments[19] and basalts[12] are consistent with this interpretation.

Oxygen isotope data

The oxygen isotope data of the pore waters (Fig. 1) indicate a gradual decrease with depth. Alteration of continental detritus > 639 m does not seem to have contributed greatly to the $\delta^{18}O$ depletion. This is particularly evident from oxygen isotope data on the 0 3–0.7-μm size fraction of quartz, which do not show any increase in $\delta^{18}O$ with depth[16] For the 60 m of authigenic basal sediments, the oxygen isotope data favour an ash precursor over a terrigenous one. Volcanic ash has a lower $\delta^{18}O$ (~ + 10‰) than terrigenous material (~ + 15‰). Thus, alteration of a given amount of ash to smectites with a $\delta^{18}O$ = + 18.7–25.1‰ (ref. 15) would lower the $\delta^{18}O$ of the pore waters more than the alteration of the same amount of terrigenous material. The major observed alteration, however, probably occurred before the resumption of sedimentation in the Miocene, as has been suggested before During the long hiatus and the period of slow accumulation of the basal sediments, all or most of the ^{18}O depletion of the pore fluids must have been obliterated by diffusional exchange with the overlying ocean. Therefore, the observed ^{18}O depletions must have occurred after the resumption of sedimentation.

The oxygen isotope depletions demand alteration of solid phases on a large scale[8]. We calculate alteration of a minimum of 20 m of basalt, 35 m of ash, or 80 m of terrigenous material at site 323 (ref. 20). Note that the order of effectiveness in producing ^{18}O depletions in the pore fluids is: basalt > ash > terrigenous material[8].

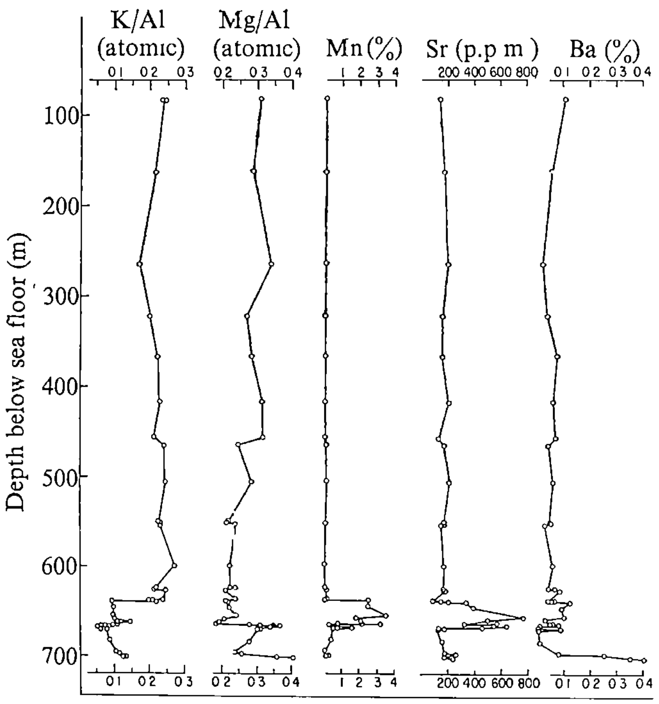

Fig. 2 Chemical composition of clay-size fraction, site 323.

From the above considerations, we conclude that the alteration reactions in the sediments cannot account for all the $\delta^{18}O$ depletion of the pore waters. The underlying basalts, however, show the presence of several authigenic minerals. Of the 28 m of basalt cored, only 10 m were recovered. Vennum[21] suggested, especially on the basis of increased drilling rates, that one or two sills may have been drilled without actually reaching oceanic basement. Increased drilling rates suggested the presence of interlayered sediments and/or highly altered basalts. We propose that much of the observed $\delta^{18}O$ depletion must have its origin in the continuing alteration in the underlying basalts.

Smectite from the basal sediments ($\delta^{18}O$ = +18.7–+25.1 ‰), as well as celadonite ($\delta^{18}O$ = +19.6‰), saponite ($\delta^{18}O$ = +24.2‰), and calcite ($\delta^{18}O$ = +26 5–+31.4‰) from the basalts formed at relatively low temperatures If alteration to these products occurred in an infinite reservoir of deep ocean water with a $\delta^{18}O$ = 0‰, the temperature range must have been 10–60 °C. Because the observed $\delta^{18}O$ values of the pore fluids are as low as −3.5‰ (Fig. 1), it is apparent that an infinite reservoir of water was probably not involved. Consequently, the alteration products may have formed over a smaller temperature range than that estimated above. The range in $\delta^{18}O$ of the calcite veins and clay minerals requires formation at different temperatures or in the presence of pore fluids with different $\delta^{18}O$ values and, therefore, at different times in the history of the site. In the basalts, an earlier formed vein ($\delta^{18}O$ = +31.4‰) is cut by a later vein ($\delta^{18}O$ = +29.5‰)[15]. It appears that the earlier vein may have formed in contact with open ocean water at 10 °C and the later vein at a higher temperature (⩽20 °C) in the presence of pore fluid with a $\delta^{18}O$ of 0‰ or lower. Such a sequence of events probably applies to the several generations of clay formation in the basalts and sediments. The hydrothermal event that caused the enrichment of transition metals and strontium 30–74 m above basement may also have been responsible for the formation of the celadonite and saponite with moderately low $\delta^{18}O$ values[12]. Based on the above oxygen isotope values, the temperature of the hydrothermal fluids could not have been > 60 °C.

Other sites

Based on chemical gradients in the pore fluids (Fig. 1), the main reaction zone in site 322 is in the lower part of the sediment section or in the underlying basalt. The $\delta^{18}O$ depletion in site 322 is very small, so that relatively little alteration appears to have occurred. Data on dissolved calcium and $\delta^{18}O$ in site 325 suggest the presence of reactive sediments below the section cored.

The significant gradients observed in the pore fluids of site 324 probably arise not only from volcanic glass alteration that has been observed in the recovered sediments of this site but also from reactions in deeper, unrecovered portions.

$\delta^{13}C$ values of -18.4 to $-21.2‰$ (PDB) of authigenic calcite in the sediments of sites 323 and 325 indicate that organic matter was the major source of carbon for these calcites[15].

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letters to nature

Transient radio source near the galactic centre

A VARIETY of interesting objects have been discovered in recent years within several arc min of the galactic centre. These include the main (east and west)[1] components of the radio source Sgr A, a compact radio source[2] at the centre of Sgr A West which may be the galactic nucleus, and a group of infrared sources[3] in a region of ~20″ diameter surrounding this compact radio source. In February 1975 Eyles *et al.*[4] discovered the transient X-ray source A1742−28 near the galactic nucleus. We report here the detection of a radio transient source which we believe is very likely to be associated with this X-ray source. Although we have evidence that this transient radio source lies close to the centre of the Galaxy, it is clearly not to be identified with the nucleus itself.

Observations of the galactic centre region have been made at intervals since June 1, 1974 using the Jodrell Bank radio-linked interferometers in a programme of investigations of the compact nuclear source. The Jodrell Bank (MK IA or MK II)-to-MK III interferometer (24-km baseline) was operated at 0.96 and 1.66 GHz while the MK IA-to-Defford interferometer (127-km baseline) was operated at 0.408 GHz. A summary of the dates of observation and of the parameters of the baselines used is given in Table 1. The observation on March 30, 1975 at 0.96 GHz revealed the emergence of a new radio source near the already known compact nuclear source. The signature of a relatively weak double source was clearly seen in the amplitude and phase data on this day; the amplitude data are reproduced in Fig. 1 along with the double source model which is the best fit to the observations. All the other observations of this region at the various times listed in Table 1 did not detect a signal at the position of the transient source and only provided upper limits to the emission at these times.

The amplitude and phase data for March 30, 1975 were used to determine an accurate offset of the transient source from the compact nuclear source whose position is given as RA = 17 h 42 min 29.291±0.005 s, dec. (1950) = −28°59′

Table 1 Radio observations of the transient source A1742−28

Date	Frequency (GHz)	Observed flux density (mJy*)	Interferometer	Projected baseline shortest ($\lambda \times 10^3$)	Projected baseline longest ($\lambda \times 10^3$)
June 01 0, 1974	1.66	< 55	MK I -MK III	60	132
November 30.6, 1974	0.408	< 50	MK I -Defford	27	86
March 30.2, 1975	0.96	220 (480†)	MK II-MK III	34	75
May 3.1, 1975	0.408	< 50	MK I -Defford	27	86
June 20.9, 1975	0.96	< 26	MK II-MK III	34	75
July 6.8, 1975	0.96	< 38	MK II-MK III	34	75
September 10.7, 1975	0.96	< 30	MK II-MK III	34	75

*1 mJy = 10^{-29} W m^{-2} Hz^{-1}.
†Flux corrected for resolution of the interferometer.

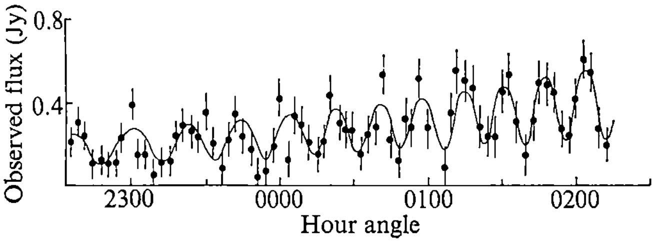

Fig. 1 The amplitude and phase plots obtained at 0.96 GHz with the MK II–MK III interferometer (24-km baseline) on March 30, 1975. The interferometer was set on the position of the compact source at the galactic nucleus (RA = 17 h 42 min 29.29 s, dec. (1950) = −28°59′17.6″). The beat pattern in the interferometer output results from the additional contribution of the new transient source. A model of a double source with a separation 53″ at p.a. = 223° is shown as a full line in the amplitude plot; the intensities of the nuclear and transient source are 0.18 and 0.39 Jy respectively; both have a Gaussian shape with a diameter between half intensity points of 1.5″.

17.6±0.1″ by Balick and Brown[2]. We have measured the offset of the transient source as 53±3″ in position angle (pa) = 223±2° which corresponds to ΔRA = −2.77±0.22 s and Δdec. = −37.4±2.8″. This places it at RA = 17 h 42 min 26.52±0.22 s, dec. (1950) = −28°59′55.0±2.8″ which lies within the larger errors of the position of the X-ray transient at RA = 17 h 42 min 26.0±4.8 s, dec. (1950) = −28°59′ 48″±1′ 12″ as shown in Fig. 2. The X-ray position uncertainty also includes the compact nuclear radio source, but it will be argued below that the radio transient source is associated with the X-ray transient.

The run of radio and X-ray intensities of the transient source are compared in Fig. 3. At radio wavelengths an upper limit is plotted for each date except the detection on March 30, 1975. At X-ray wavelengths[5] the emission decayed rapidly for 40 d with an e-folding time of 12 d followed by a slower decay for 140 d with an e-folding time of 90 d. It is of course not known where, between February 22 and March 30, the maximum in the radio emission occurred. Figure 3 shows, however, that the radio decay does not mirror the X-ray decay; between March 30 and May–July the X-ray intensity falls only by a factor of ~ 2 while the radio has fallen by a factor of ≳ 6. Additional upper limits to the X-ray emission outside the time of the main transient activity have been supplied by Dr P. W. Sanford of the Mullard Space Science Laboratory and Dr M. J. L. Turner of Leicester University.

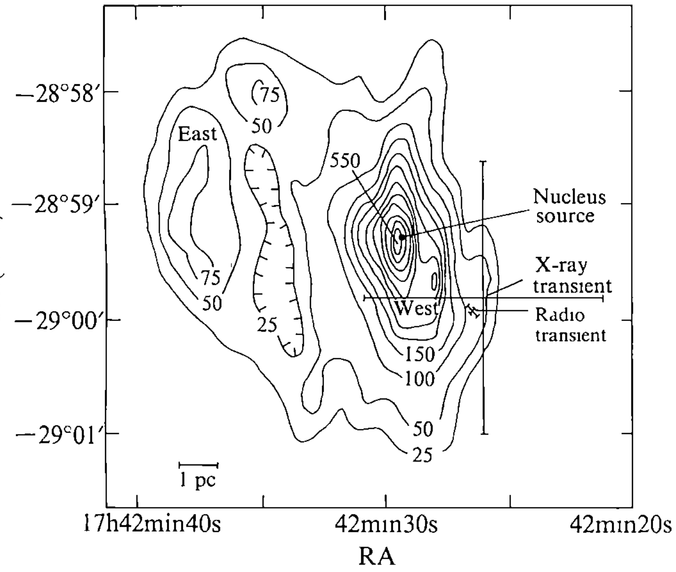

Fig. 2 A 5.0-GHz map of the Sgr A area by Ekers *et al.* showing the position of the nuclear source (filled circle) and the new transient source (cross); the size of the cross indicates the position uncertainty of the transient source. The position and position error of the X-ray transient source A1742−28 as given by Eyles *et al.* are indicated as a large cross.

There are two arguments which suggest an association between the radio and X-ray transients. The first is that, in spite of the sporadic nature of the radio and X-ray observations, there is general agreement in the period of the activity at both radio and X-ray wavelengths; at times beyond ±40 d of the X-ray eruption there was no detectable radio and only weak X-ray emission (Fig. 3). The second argument is the close agreement to better than 7″ in each coordinate between the measured radio and X-ray position of the transient objects. This implies that liberal systematic errors were assigned by Eyles *et al.*; the true error may have been composed only of their random error of 10″.

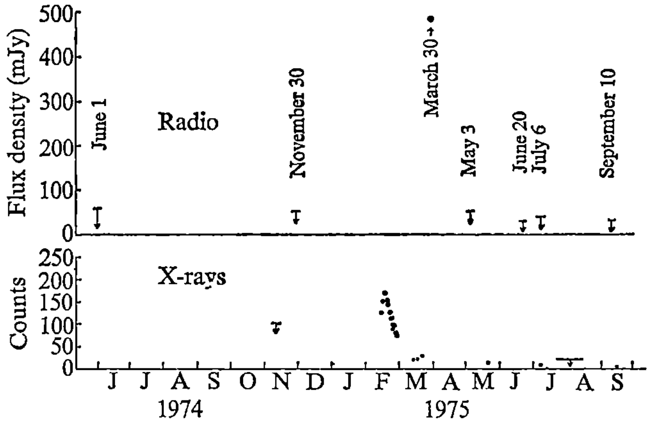

Fig. 3 A plot of the observations of the X-ray and radio intensity of A1742−28 as a function of time. The frequencies of the radio observations are given in Table 1.

The interferometric data show that the angular diameter of both the transient source and the nuclear source are 1.5±0.3″ at 0.96 GHz. The true flux of the source on March 30, 1975 was 0.48±0.07 Jy when corrected for the resolution of the interferometer. Available information on the diameter of the nuclear source as a function of wavelength, λ, indicates that its diameter increases as λ^2; this is the dependence expected for interstellar scattering. The high electron line-integral between the Sun and the galactic centre can readily account for the observed scattering. The close similarity in the angular sizes of the transient and nuclear sources which lie so close together in the sky would argue that their diameters have been affected by the same clouds of interstellar electrons presumably lying near the galactic nucleus. We therefore infer that the transient source is also at the galactic centre. It is of interest to note that the radio outburst of Cyg X-3 was also broadened (to a half power width of 2″) by interstellar scattering at 0.408 GHz (ref. 6). Cyg X-3 is at a distance of ~ 10 kpc also and lies behind the Cyg X radio H II region; its intrinsic diameter is ≲ 0.1″ (refs 6 and 7).

The present observations provide strong evidence that the radio and X-ray sources are related and that they lie 53±3″ from the true galactic nucleus as defined by the compact radio source. Although the transient source lies within the outer contours of the Sgr A West radio source, there seems to be no structural feature in the 5.0 GHz map of Ekers *et al.*[1] (Fig. 2) which can be identified with it; nor has any intense infrared source been reported[8] at this position although it lies on the infrared ridge at 2.2 μm. If it is at a distance from the centre of 2.5 pc which corresponds to its projected angular distance then it is within a region of high stellar density of $10^5 M_\odot \mathrm{pc}^{-3}$ in the model of Oort[9]; this density is a factor of 10^6 greater than in the solar vicinity. One of the stars in this nuclear concentration is presumably responsible for the radio and X-ray outburst. Braduardi *et al.*[5] suggest that it may be a binary system containing a compact object with variable mass transfer because of its similarity with 3U1543−47.

The X-ray and radio properties of this transient object do not seem to be unique even though it is occurring in what might be considered a unique part of the Galaxy. Its intrinsic radio luminosity (5×10^{33} erg^{-1} between 1 and 100 GHz if assumed to have a flat spectrum) lies between that of bursts observed in Cyg X-1 and Cyg X-3. The apparent association in time between outbursts or changes in intensity at radio and X-ray wavelengths observed here is also found in Cyg X-1 and the new transient source A0620−00 (refs 10, 11). The intrinsic peak X-ray luminosity of 3×10^{38} erg^{-1}, however, is at the top end of the range measured for known X-ray sources[4,5], if it is placed at the galactic centre, as the present results indicate.

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Steepening of the γ-ray background spectrum from local γ-ray production

Because the Solar System lies in a spiral arm of our Galaxy, much of the γ-ray flux arriving at large angles to the galactic plane is of local origin. We have tried to calculate this contribution to the flux, and find that the effect is particularly pronounced for γ rays of energy > 100 MeV. This implies a steeper spectrum for extragalactic γ rays than has been hitherto expected.

Beside γ radiation originating from the Galaxy there is evidence for a general γ background. The differential energy spectrum of this diffuse radiation can be represented by a power law:

$$dJ/dE_{\gamma} = (2.7\pm0.5)\times10^{-7}\times$$
$$\times(E_{\gamma}/100)^{-2.4\pm0.2}\ \text{photons cm}^{-2}\,\text{s}^{-1}\,\text{sr}^{-1}\,\text{MeV}^{-1}$$

for photon energies between 35 and 170 MeV (ref. 1). Beside these results from the SAS-2 satellite experiment there are results from balloon experiments covering the range 1–100 MeV which show that this diffuse—for lower energies highly isotropic—radiation has an intensity above the line extrapolated from high energy X-ray data. The most prominent of the models proposed to explain this are (1) the decay of neutral pions produced by cosmic-ray interactions with the intergalactic gas at high redshifts (protar hypothesis)[2], (2) the decay of neutral pions arising from matter–antimatter annihilation[3] and (3) pair production by cosmic-ray protons on the microwave background followed by an electromagnetic cascade involving the inverse Compton effect[4]. These all favour an extragalactic origin of the radiation.

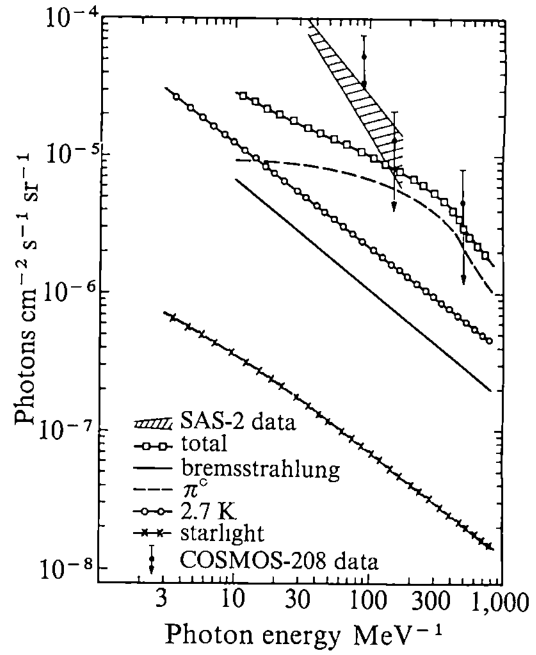
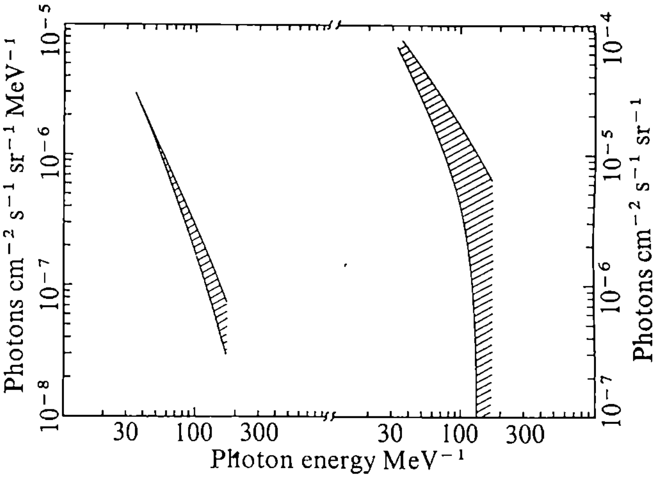


Fig. 1 Integral spectral energy spectrum of galactic γ rays from directions with high galactic latitude ($|b^{II}|>30°$) in comparison with SAS-2 and COSMOS-208 data The half thickness of the relativistic electron disk has been taken as $z_B=1$ kpc.

Usually this diffuse background radiation is observed by pointing the detector axis away from the galactic plane in directions with high galactic latitude b^{II}. In the SAS-2 experiment all γ rays coming from regions with $|b^{II}| > 30°$ have been attributed to the diffuse component. On the other hand, studies of starlight polarisation, Faraday rotation of pulsars and extragalactic radio sources[5] and 21-cm line emission indicate that the Solar System is located inside a spiral arm of our Galaxy implying that even at high latitudes a part of the observed γ rays are produced in the local spiral arm. To estimate the contribution of these locally produced γ rays from high latitudes we calculate the differential and integral spectral flux from directions observed by SAS-2, namely I ($l^{II}=0°$, $b^{II}=58°$), II ($l^{II}=285°$, $b^{II}=75°$) and III ($l^{II}=300°$, $b^{II}=-45°$), by taking into account bremsstrahlung[6] and π^0-decay[7] in the

Fig. 2 Corrected ($z_B=1$ kpc) differential (left) and integral (right) energy spectrum of extragalactic γ rays.



interstellar gas and inverse Compton scattering of low energy starlight and black-body photons[8] as well as the final angular resolution of the SAS-2 γ-ray detector which was treated as a two-dimensional Gauss function with a s.d. of 5°.

The astrophysical parameters contained in the emissivities of the various γ-ray production processes were calculated by using analytical formulae for the latitudinal distribution of stellar and interstellar matter[9,10]. The most important derived parameters are the hydrogen column densities in the three directions I, II and III, which are 5.8×10^{20}, 5.0×10^{20} and 8.3×10^{20} atoms cm^{-2} respectively, the mean value of which, $N_{\text{H}}=6.4\times10^{20}$ H atoms cm^{-2}, determines the absolute values of the π°-spectrum which, as can be seen from Fig. 1, is the main part of the total calculated spectrum for photon energies > 100 MeV. Checking these column densities with estimates from Lyman α absorption measurements[11,12] ($N_{\text{H}}\simeq3\times10^{20}$ H atoms cm^{-2})—which provide only lower limits because of unseen gas beyond the observed stars—interstellar reddening data[13] (N_{H} between 2×10^{20} and 9×10^{20} H atoms cm^{-2}) and 21-cm observations[14] applying a factor 2 for the abundance of hydrogen molecules[15–17] ($N_{\text{H}}\simeq8\times10^{20}$ H atoms cm^{-2}) we find reasonable agreement. The local energy spectrum of cosmic ray protons and α-particles is contained in the production rate of the π°-decay[7]; the interstellar electron spectrum has been taken as a power law with a spectral index 1.8 for energies < 2 GeV and 2.5 for energies > 2 GeV (refs 18 and 19).

It can be seen from Fig. 1 that for photon energies > 100 MeV the locally produced γ-ray component becomes dominant: at least we find that $51^{+21}_{-14}\%$ of the integral (>100 MeV) spectral flux is of galactic origin where it should be noted that the error bars come from the uncertainties in the SAS-2 measurements. On the other hand at lower energies ($E_{\gamma}>40$ MeV) only $21^{+4}_{-2}\%$ of the integral spectral flux is produced in the local environment of the Sun If the upper limit at 500 MeV measured in the COSMOS-208 experiment[20] will be confirmed by future measurements we can conclude that at these energies the γ-ray flux from high latitudes arises predominantly from local events. Repeated calculations with different values of the half-thickness of the relativistic electron disk $z_{\text{B}}=300$ pc and 500 pc lead to the same conclusion because only the black-body component—which has no significant influence on the upper part of the total spectrum—depends linearly from the values of z_{B}.

This effect of the increasing importance of locally produced γ-rays can also be seen by studying the ratio of the local and empirical differential spectral flux: at 40 MeV 6%, at 100 MeV $16^{+4}_{-2}\%$ and at 170 MeV $32^{+12}_{-6}\%$ are of galactic origin. In terms of the 'real' extragalactic γ radiation this means that the high energy end of the measured spectra must be corrected because of local events whereas at lower energies the measured spectra are not affected by galactic γ-ray production. This last result is in good agreement with isotropy measurements of this diffuse radiation showing that at low energies (<10 MeV) > 87% of the measured flux is of extragalactic origin[21].

In principle, by subtraction of calculated locally produced γ rays from the observed spectrum at ~ 100 MeV, one may obtain the 'real' extragalactic γ-ray spectrum, the discussion of which should make possible a distinction between the annihilation hypothesis and the protar hypothesis. The former gives rise to a steeper spectrum than predicted by the latter hypothesis[3]. We are quite aware of the fact that statistics of the SAS-2 as well as the COSMOS-208 data above 100 MeV are insufficient for conclusive arguments concerning these two alternatives. Nevertheless, to get a tentative result we have made this subtraction shown in Fig. 2 which seems to be more in favour of the annihilation hypothesis rather than the protar hypothesis It will be most important to obtain further measurements to get better statistics on the energy spectrum > 100 MeV from high latitude directions as well as on the isotropy of arrival directions in this energy range.

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Particle creation and Dirac's large numbers hypothesis

IN connection with cosmologies based on the large numbers hypothesis[1] (LNH), Dirac[2] has suggested that continuous creation of matter is required. Two modes of particle creation have been proposed and cosmological models corresponding to each mode have been studied[2]. We demonstrate here that, within the context of the LNH, the number of particles in the Universe varies as expected ($N\propto t^2$) so that particle creation is unnecessary. Some unpleasant features of cosmologies based on the LNH are also discussed.

To appreciate fully the results to be presented for cosmologies based on the LNH, it will be valuable to review first the corresponding relationships in conventional cosmologies in which the gravitational constant, G, does not vary with epoch. For such models, the variation of the density with the age of the Universe is given by

$$G\rho t^2 = \text{constant} \tag{1}$$

This result is valid for early times (high densities); it is also valid now if the density is equal to the critical density ($\rho_c = 3H_0{}^2(8\pi G)^{-1}$). Now, we will be interested in the variation with epoch of the number of particles in the Universe. By "in the Universe" we mean within the horizon or, up to a numerical factor, within the Hubble distance c/H. The volume of the Universe thus varies with epoch as $V\propto H^{-3}\propto t^3$ (see, for example, ref. 3). For a pressure-free (matter dominated (MD)) cosmological model, the particle density, n, is proportional to the density ρ. Then, from equation (1) we see that $n\propto\rho\propto t^{-2}$ so that the number of particles in the Universe increases as

$$N = nV \propto t^{-2}t^3 \propto t \tag{2}$$

The presence of the black-body radiation suggests that pressure played an important dynamical role in the early Universe. If the early evolution was radiation dominated (RD), then $n\propto\rho^{3/4}\propto t^{-3/2}$. In this case the number of particles in the Universe varies with epoch as

$$N = nV \propto t^{-3/2}t^3 \propto t^{3/2} \tag{3}$$

We will also be interested in the variation with epoch of the temperature, T, of the background radiation. Since the temperature varies with particle density as $T \propto n^{1/3}$, it follows that

$$\begin{aligned} \mathrm{MD} &: T \propto n^{1/3} \propto \rho^{1/3} \propto t^{-2/3} \\ \mathrm{RD} &: T \propto n^{1/3} \propto \rho^{1/4} \propto t^{-1/2} \end{aligned} \qquad (4)$$

A reasonably accurate $t(T)$ relationship may be obtained if account is taken of the fact that the Universe is MD for $T \lesssim 10^4\, T_0$ and RD for $T \lesssim 10^4\, T_0$ ($T_0 \simeq 2.7$ K).

$$\begin{aligned} \mathrm{MD} &: t \simeq 10^{18}\, T^{-3/2}\ \mathrm{s} \\ \mathrm{RD} &: t \simeq 10^{20}\, T^{-2}\ \mathrm{s} \end{aligned} \qquad (5)$$

Recall for future reference that primordial nucleosynthesis occurs when $10^8 \lesssim T/T_0 \lesssim 10^9$ at which time the age of the Universe is $10 \lesssim t(\mathrm{s}) \lesssim 10^3$ (see, for example, ref. 4).

We now turn to a re-examination of these results in a cosmology based on Dirac's large numbers hypothesis[1]. Dirac noticed that the ratio of the present age of the Universe (t_0) to an atomic unit of time ($t_e \equiv e^2/m_e c^3 \simeq 10^{-23}$ s) is a large dimensionless number (LDN).

$$\tau_0 \equiv t_0/t_e \simeq 10^{40} \qquad (6)$$

In the past (future) this ratio had (will have) a different value; $\tau \equiv t/t_e$ varies with epoch. Dirac was led to suggest that all such LDN vary with epoch; if the present value of a LDN is $N_0 \simeq \tau_0^{\alpha}$ then, $N \propto \tau^{\alpha} \propto t^{\alpha}$. One of the most striking consequences of this assumption is that the gravitational 'constant', G, must vary with time[1,2]: $G \propto t^{-1}$. An immediate consequence for cosmologies based on the LNH is that: $\rho \propto t^{-1}$. This rapid variation of epoch with density ($t \propto \rho^{-1}$ compared with $t \propto \rho^{-1/2}$ previously) has far-reaching effects to which we now turn.

The number of observable particles in the Universe is an example of a LDN. At present, $N_0 \simeq \tau_0^2$, so that the LNH requires that $N \propto t^2$. This is a more rapid variation of N with t than given by equation (2) and Dirac[2] was led to conclude that particle creation was necessary. (It should be noted that Jordan[5] also proposed a cosmology based on the LNH. He noted that $G \propto t^{-1}$ in such cosmologies and for the reasons just outlined concluded that particle creation was required.) The result contained in equation (2) depends crucially, however, on the assumption of a constant G. If $G \propto t^{-1}$ we obtain for a MD cosmology

$$N = nV \propto \rho V \propto t^{-1} t^3 \propto t^2 \qquad (7)$$

This result is in perfect agreement with the LNH; particle creation is unnecessary. Note that for an RD model a somewhat different result is obtained

$$N = nV \propto \rho^{3/4}\, V \propto t^{-3/4} t^3 \propto t^{9/4} \qquad (8)$$

Although in this case the particle number increases more rapidly than t^2, we will not speculate whether the difference in exponent between 2 and 9/4 is sufficiently significant to require particle destruction!

Next, let us consider the relationship between age and temperature in cosmologies based on the LNH. For such cosmologies the variation of t with T is much more rapid than in conventional cosmologies (compare with equations (4) and (5))

$$\begin{aligned} \mathrm{MD} &: T \propto n^{1/3} \propto \rho^{1/3} \propto t^{-1/3} \\ \mathrm{RD} &: T \propto n^{1/3} \propto \rho^{1/4} \propto t^{-1/4} \end{aligned} \qquad (9)$$

As before, if account is taken of the relative periods of time that the Universe is MD and RD, we obtain

$$\begin{aligned} \mathrm{MD} &: t \simeq 10^{18.5}\, T^{-3}\ \mathrm{s} \\ \mathrm{RD} &: t \simeq 10^{23}\, T^{-4}\ \mathrm{s} \end{aligned} \qquad (10)$$

These extremely strong dependences of t on T have several unfortunate consequences. We noted earlier that primordial nucleosynthesis occurs when $10^8 \lesssim T/T_0 \lesssim 10^9$. It follows from equation (10) that the age of the Universe is only $\sim 10^{-15}$ s when $T \simeq 3 \times 10^9$ K and, by $t \simeq 10^{-11}$ s the temperature has dropped to $T \simeq 3 \times 10^8$ K. Clearly there is no time available for any nuclear reactions to proceed. There is apparently no nucleosynthesis at all in cosmologies based on the LNH. In such models, then, the observed large and uniform abundance of helium[6] is without explanation.

Another unpleasant aspect of the rapid variation of t with T is the extremely steep decline in the number of particles in the Universe with increasing temperature

$$\begin{aligned} \mathrm{MD} &: N \simeq 10^{78}\, (T_0/T)^6 \\ \mathrm{RD} &: N \simeq 10^{90}\, (T_0/T)^9 \end{aligned} \qquad (11)$$

As a result, for $T \gtrsim 10^{10}\, T_0$ there is less than one particle in the Universe! Clearly, the usual assumptions of homogeneity will have broken down.

Finally, consider the scattering of photons by electrons in the early Universe. The number of scatterings per s is

$$\tau_\gamma^{-1} = n\sigma_{\gamma e} \simeq 10^{-20}\, T^3\ \mathrm{s}^{-1} \qquad (12)$$

Comparing equations (10) and (12) it follows that the total number of scatterings is $t/\tau_\gamma \ll 1$. Thus, there is no way to establish thermodynamic equilibrium between the photons and electrons and so the black-body spectrum of the background radiation is without explanation.

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Gravitational analogue of magnetic force

THE gravitational analogue of the magnetic force has been discussed[1,2] within the framework of special relativity. The difference of opinion between these authors stems basically from the fact that a rigorous calculation of this force must be carried out using the general theory of relativity, and we give here such a calculation.

Consider two massive charged particles with masses, charges, coordinates and velocities, denoted by m_i, e_i, r_i and v_i, respectively ($i = 1, 2$). Then, to order c^{-2}, the Lagrangian for the system is[3]

$$L = L_0 - V \equiv L_0 - (V_{\mathrm{EIH}} + V_{\mathrm{D}} + V_{\mathrm{M}}) \qquad (1)$$

where $L_0 - V_{\mathrm{EIH}}$ is the Einstein–Infeld–Hoffmann Lagrangian for uncharged particles[4], $L_0 - V_{\mathrm{D}}$ is the Darwin Lagrangian of classical electrodynamics for a system of two charges (see ref. 4, p. 168) and V_{M} consists of mixed mass–charge terms.

Explicitly, we have

$$L_0 = \tfrac{1}{2}m_1v_1{}^2 + \tfrac{1}{2}m_2v_2{}^2 + \frac{1}{8c^2}(m_1v_1{}^4 + m_2v_2{}^4) \qquad (2)$$

$$-V_{\mathrm{EIH}} = \frac{Gm_1m_2}{r} - \frac{Gm_1m_2}{2rc^2}[\mathbf{v}_1\cdot\mathbf{v}_2 + (\mathbf{v}_1\cdot\mathbf{n})(\mathbf{v}_2\cdot\mathbf{n}) - 3(\mathbf{v}_1 - \mathbf{v}_2)^2]$$

$$- \frac{G^2m_1m_2(m_1+m_2)}{2r^2c^2} \qquad (3)$$

$$-V_{\mathrm{D}} = -\frac{e_1e_2}{r} + \frac{e_1e_2}{2rc^2}[\mathbf{v}_1\cdot\mathbf{v}_2 + (\mathbf{v}_1\cdot\mathbf{n})(\mathbf{v}_2\cdot\mathbf{n})] \qquad (4)$$

and

$$-V_{\mathrm{M}} = \frac{G}{2r^2c^2}[2e_1e_2(m_1+m_2) - (e_1{}^2m_2 + e_2{}^2\,m_1)] \qquad (5)$$

where $\mathbf{n}$ is a unit vector along $\mathbf{r}_1 - \mathbf{r}_2$.

The existence of a gravitational analogue of the magnetic force (the Gv_iv_j terms) is now apparent. It is instructive, however, to consider, as in refs 1 and 2, the two particles to be in equilibrium in their rest frames, from the balance of Newtonian and Coulomb forces. Then, we consider a frame of reference in which the particles, while maintaining the same distance apart, are moving with uniform velocity $\mathbf{v}_1 = \mathbf{v}_2 \equiv \mathbf{v}$ with $\mathbf{v}_1\cdot\mathbf{n} = \mathbf{v}_2\cdot\mathbf{n} = 0$. Thus

$$L_0 = \tfrac{1}{2}(m_1+m_2)v^2 + \frac{v^4}{8c^2}(m_1+m_2) \qquad (6)$$

$$-V_{\mathrm{EIH}} = \frac{Gm_1m_2}{r}\left(1 - \frac{v^2}{2c^2}\right) - \frac{G^2m_1m_2(m_1+m_2)}{2r^2c^2} \qquad (7)$$

and

$$-V_{\mathrm{D}} = -\frac{e_1e_2}{r}\left(1 - \frac{v^2}{2c^2}\right) \qquad (8)$$

The corresponding 'generalised force' on particle 1 from particle 2 is

$$F = \left|\left(\frac{\partial L}{\partial \mathbf{r}_1}\right)\right| = -\frac{Gm_1m_2}{r^2}\left(1 - \frac{v^2}{2c^2}\right) + \frac{G^2m_1m_2(m_1+m_2)}{r^3c^2}$$

$$+ \frac{e_1e_2}{r^2}\left(1 - \frac{v^2}{2c^2}\right) - \frac{G}{r^3c^2}\left(2e_1e_2(m_1+m_2) - (e_1{}^2m_2 + e_2{}^2m_1\right) \qquad (9)$$

Thus, the condition for equilibrium in the moving frame is $e_i = \pm G^{1/2}m_i$. In other words, as previously noted[5,6] we have the same condition for equilibrium in the general relativistic case as in the Newtonian case. In addition, we notice that the gravitational analogue of the magnetic force, in this particular example, is simply obtained from the magnetic force $-e_1e_2v^2/2r^2c^2$ by the replacement $e_1e_2 \rightarrow -Gm_1m_2$ and, to lowest order in (v/c), this agrees with the result given in ref. 1. On examination of equations (3) and (4), however, it is clear that this is not true in general.

In fact, it is now clear that arguments based on the use of special relativity only[1,2] are inadequate in two major respects: (1) They fail to uncover the existence of various higher-order terms (in addition to the Gv^2 terms, the so-called gyron force[1] terms), such as the purely gravitational G^2 terms (which, at least for bound-state problems—perihelion precession of Mercury—are comparable in magnitude to the Gv^2 terms), nor the mixed Ge_ie_j terms. The existence of these nonlinear terms is intimately related to a well-known facet of general relativity theory—that the electromagnetic field and the gravitational field itself both act as sources of the gravitational field. (2) They provide no unambiguous way of deciding on the correct law of transformation of forces, whereas such a transformation emerges naturally in the general relativistic approach.

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Dispersion and the transverse aether drag

THERE has been controversy about the correct expression of the displacement, d, introduced by the transverse motion of a dispersive dielectric slab on a light beam. Player[1] proposed the formula $d = vt(n_g - n^{-1})/c$, where v is the slab transverse velocity, t the slab thickness, n_g and n the group and wave refractive indices of the medium, respectively, and c the velocity of light in free space. This expression has been verified in very careful experiments reported by Jones[2]. I point out here that a simple model demonstrates that (in agreement with Player's formula) d must vanish when $v_gv_\phi = c^2$, where $v_g \equiv c/n_g$ is the group velocity and $v_\phi \equiv c/n$ is the phase velocity in the slab.

Let the slab be cut out from a set of plane parallel perfectly conducting surfaces. In other words, instead of considering a real dielectric, we consider a set of perfectly conducting sheets in air. Such artificial dielectrics are commonly used at microwave frequencies. The direction of the incident ray, the direction of the incident polarisation (E field), and the direction of the motion are assumed to be in the plane of these surfaces (see Fig. 1). The propagation of electromagnetic waves in the artificial dielectric slab just described is isotropic in the plane of the surfaces and satisfies the well known relationship $v_gv_\phi = c^2$. On the other hand, the motion of perfectly conducting surfaces in their own plane is immaterial. Therefore the transverse displacement d of the light beam must be equal to zero when the relation $v_gv_\phi = c^2$ holds.

The same conclusion can be reached by considering a particle with non-zero rest mass, such as an electron, traversing a set of perfectly transparent grids at different potentials[3]. As is well known, the de Broglie matter wave associated with the electron satisfies the relationship $v_gv_\phi = c^2$, where the group velocity v_g can be identified with the electron velocity. Here

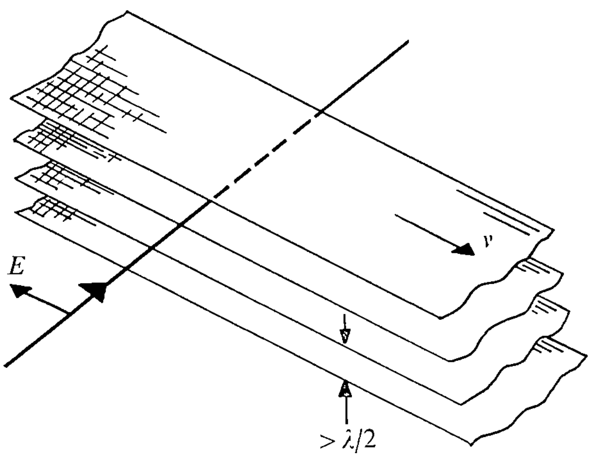

Fig. 1 Gedanken apparatus, consisting of plane parallel conducting sheets moving (velocity **v**) transverse to incident radiation

again, it can be seen that a motion of the grids in their own plane is immaterial and should not affect the electron trajectory.

My arguments, of course, do not prove Player's formula, but they prove that alternative general formula that do not satisfy the requirement set up above are incorrect. Note that the condition $d=0$ when $v_g v_\varphi=c^2$ should hold also for light beams at non-normal incidence, a case that was not considered explicitly by Player.

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Tin mineralisation and mantle hot spot activity in south-eastern Missouri

SILLITOE has pointed out interesting relationships between tectonic setting and a particular class of tin mineralisation[1]. Typical mineralisation in these deposits comprises disseminated cassiterite accompanied by topaz, lithia mica, and wolframite in greisens and quartz veins above small subvolcanic alkaline and peralkaline granite plutons which lack demonstrable affiliation with contemporary plate boundaries.

I here describe briefly the tin mineralisation and petrologic–tectonic setting of the Saint Francois Mountains volcanic–plutonic terrain of south-eastern Missouri, which may possibly represent the oldest example of this class of mineral deposit.

The Saint Francois Mountains form a distinctive unmetamorphosed igneous complex comprising chiefly alkalic to peralkalic rhyolite and granite, associated with a major negative gravity anomaly[2]. The plutons seem to be more or less concordant injections into chemically-related rhyolitic ignimbrite and breccia sheets[3] The date of this volcanic–plutonic episode is well established by recent detailed geochronologic studies[4] at about 1,500 Myr BP The effusive rocks range in composition from andesite to rhyolite, whereas the plutonic rocks vary in composition from granodiorite to granite and minor syenite. The volcanic series and the plutonic series are each volumetrically dominated by the high-silica end members[3]. Porphyritic textures are widespread in many of the intrusive bodies; the granites locally display the well developed rapakivi texture[5] characteristic of anorogenic granites throughout the world[6]. Palaeozoic strata cover the country rock which surrounds the igneous exposures of the Saint Francois Mountains, but drill-core studies indicate the 1,500-Myr-old rocks were intruded into and erupted on an older metamorphic–plutonic terrain[7]. The exposed volcanic–plutonic complex is cut by diabase dykes and layered gabbro sills of later, but uncertain, Precambrian age[8].

The Precambrian volcanic rocks of south-eastern Missouri have long been known for their association with magmatic iron ores[9–11]. Tin mineralisation of subeconomic grade, however, is associated with the contact between rhyolite roof rocks and the intruding granite at a locality known as the Silver Mine (90.5°W; 37.5°N). The roof-zone greisen deposits of that locality have been sporadically worked for silver and tungsten during the past 100 yr. Mineralisation in the Silver Mine area consists of well-defined quartz veins containing disseminated cassiterite, lithia mica (zinnwaldite?), topaz, fluorite, wolframite (hubnerite), arsenopyrite, pyrite, chalcopyrite, sphalerite, and argentiferous galena[12]. Major, trace, and rare earth element distributions in the veins and altered wallrocks indicate extensive replacement of the host granite by high temperature, fluorine-rich solutions[13] Original feldspars in the wallrock adjacent to the veins have been completely destroyed and supplanted by an assemblage of topaz, quartz, fluorite, sericite, lithia mica, and cassiterite[13]. Beryl and tourmaline are not present in the veins or the well defined vein envelopes of sericitic wallrock alteration[13]. This mineral assemblage and its petrologic setting are remarkably similar to those of the Younger Tin Fields of Nigeria[14] and the tin deposits of Rondônia, Brazil[15].

The tin mineralisation in south-eastern Missouri differs in major ways from the well known tin deposits of Bolivia and Japan. The latter are both located in calc-alkaline igneous settings and are thus apparently related to convergent plate processes. Sillitoe *et al*[16] have described the Bolivian tin deposits as porphyry type mineralisation characterised by pervasive sericitic alteration, stockworks of veinlets, and widespread hydrothermal intrusion breccias—features entirely lacking in the Saint Francois Mountains deposit. The tin mineralisation of Missouri bears a resemblance to the subvolcanic (xenothermal) deposits of Japan[17] which also contain topaz and lack both tourmaline and beryl The Japanese ores, however, contain tin minerals other than cassiterite (for instance, stannite, stannoidite, and mawsonite), primary bornite and chalcocite, native silver, and silver sulphosalts in a gangue which includes apatite, rhodochrosite, and orthoclase[17]. None of these minerals has been reported at the Silver Mine workings[12]. Lithia mica is present in Japanese hypothermal tin veins but is not found in the subvolcanic (xenothermal) deposits[17]. In the Andes of South America, subduction processes have resulted in a definite zoning of metal provinces wherein iron mineralisation and tin–molybdenum mineralisation are widely separated in space[18]. The spatial coincidence of south-eastern Missouri iron and tin mineralisation does not support the hypothesis that there is a former subduction zone beneath the Precambrian igneous terrain of the region.

The hypothesis that tin-bearing alkaline igneous complexes are generated above mantel hot spots before the formation of triple junctions and associated rifts[1] is supported by geological and geophysical relationships in south-eastern Missouri. Burke and Dewey[19] have interpreted the Mississippi Embayment as a failed arm (aulacogen) of a late Palaeozoic triple junction centred near Jackson, Mississippi, but there is evidence that this event was preceded by a major period of late Precambrian rifting in central North America which commenced about 1,300 Myr BP (refs 20–22).

Tin-bearing subvolcanic complexes in Nigeria, Rondônia, and Namibia occur in elongated belts 300–400 km long[1].

Deep drilling of the Mid-continent Precambrian terrain has returned rock samples which are similar in petrography and general age to those exposed in the Saint Francois Mountains. These samples apparently represent buried subvolcanic complexes, similar to those exposed in south-eastern Missouri, which are arranged in a north-east–south-west trending belt[4]. Data from Muehlberger *et al.*[23] suggest that the age of the complexes becomes progressively younger south-westward, inviting the speculation that the belt records a period of north-eastward plate movement over a mantle hot spot which supplied tin, fluorine, and other mantle-derived elements to rising magmas. It must be noted however, that the only tin deposit at present known to exist in this belt of subvolcanic complexes is that exposed in the Saint Francois Mountains.

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The stability of the earth's core and the geodynamo

RECENT work[1,2] has indicated that precession fails by at least an order of magnitude to provide the power necessary to drive the geodynamo. It is therefore necessary to consider again thermal convection in the Earth's core, which we do in this paper.

Thermal convection requires that the fluid core be unstable in the radial direction. A fluid element displaced radially from the Earth's centre must find itself surrounded by fluid of greater density so that it will continue to rise. If this is not the case the fluid element will either remain in its new position (neutral stability) or suffer a restoring force towards its initial position. The density distribution $\rho = \rho(r)$ which determines the stability of the core will depend on two thermodynamic variables such as pressure p and entropy s, together with as many parameters as there are components of the core fluid. In any homogeneous region in which changes of density are due to adiabatic compression alone, the density distribution is given by the Adams–Williamson equation

$$\frac{d\rho}{dr} = \frac{-g\rho^2}{k_s} = \frac{-g\rho}{\varphi} \tag{1}$$

where g is the acceleration due to gravity and k_s the adiabatic incompressibility defined by the equation

$$\frac{1}{k_s} = \frac{1}{\rho}\left[\frac{\partial\rho}{\partial p}\right]_s$$

The parameter

$$\varphi = \frac{k_s}{\rho} = V_P^2 - \frac{4}{3}V_S^2 \tag{2}$$

where V_P and V_S are the velocities of P and S waves and are known from seismology. This distribution of density is neutrally stable since the change from the adiabatic expansion suffered by an element of fluid displaced radially outward is exactly equal to the change in density of the surrounding fluid over this displacement.

Suppose now that the density distribution depends not only on adiabatic compression but also on temperature (or entropy). Then, for a homogeneous medium

$$\frac{d\rho}{dr} = \left[\frac{\partial\rho}{\partial p}\right]_s \frac{dp}{dr} + \left[\frac{\partial\rho}{\partial s}\right]_p \frac{ds}{dr} \tag{3}$$

The first term on the right hand side is the Adams–Williamson density gradient and the second the contribution from the change in density with entropy. If the entropy is constant throughout the fluid so that $ds/dr = 0$, equation (3) reduces to equation (1) and the density gradient is uniquely determined. This, however, is very unlikely since it implies that the temperature gradient dT/dr is exactly equal to the adiabatic gradient $g\alpha T/c_p$ throughout the core since

$$\left[\frac{\partial\rho}{\partial s}\right]_p \frac{ds}{dr} = \alpha\rho\left[-\frac{dT}{dr} - \frac{g\alpha T}{c_p}\right] \tag{4}$$

where α is the volume coefficient of expansion and c_p the specific heat at constant pressure.

Birch[3] has written equation (3) in the alternative form

$$\frac{d\rho}{dr} = \frac{-g\rho}{\varphi} + \alpha\rho\tau \tag{5}$$

where τ is the excess of the actual temperature gradient over the adiabatic gradient. A necessary condition for convection to be possible in any stationary layer is that $\tau > 0$. Various estimates of τ have been made in the past with differing conclusions.

An estimate of the term $\alpha\rho\tau$ in the Earth's core may, in principle, be obtained from an independent evaluation of the actual density gradient and that given by the Adams–Williamson equation. Such an approach is inherent in the work of Pekeris and Accad[4] who wrote

$$\alpha\rho\tau = \frac{g\rho\beta}{\varphi} \tag{6}$$

so that

$$\beta = \beta(r) = 1 + \frac{\varphi}{g\rho}\frac{d\rho}{dr} \tag{7}$$

They chose constant values of β (−0.2, 0 and 0.2) throughout the core and compared the observed free oscillation periods of the Earth with those calculated from their estimate of β. A programme to try to determine the behaviour of β in the Earth's core has been undertaken. Preliminary calculations show that the extreme values ± 0.2 are physically unrealistic and that $|\beta|$ must be much smaller. In some models of the Earth, however, the density stratification changes from stable ($\beta < 0$) in one part of the core to unstable ($\beta > 0$) in another.

It can be seen that β may be estimated (from equation (7)) throughout the outer core without any knowledge of the temperature gradient. It is very sensitive, however, to the variation of the density gradient $\frac{d\rho}{dr}$. Crossley (personal communication) has estimated β for a number of Earth models, and obtained values lying in general between about −0.2 and 0.2, although some models gave values outside this range over part of the core. Knowing β, the excess τ of the actual temperature gradient over the adiabatic temperature gradient may be estimated from equation (6). Estimates of τ are more unreliable because of uncertainties in the values of α in addition to those in β. Frazer[5] has examined the literature and found that estimates of α range from 3.6×10^{-6} to 10×10^{-6} K^{-1}. It is likely that α will be pressure dependent and Jacobs[6] suggested that there may be a linear relationship between 1/α and pressure in analogy with Bullen's[7] compressibility–pressure hypothesis. He obtained values of α of 9.3×10^{-6} K^{-1} at the core–mantle boundary and 4.4×10^{-6} K^{-1} at the inner core–outer core boundary—both these values of α lie within the range of those given by Frazer.

The adiabatic gradient may, alternatively, be written in terms of Grüneissen's 'constant' γ

$$\frac{dT}{dr} = \frac{-g\alpha T}{c_p} = \frac{-g\gamma T}{\varphi} \tag{8}$$

γ was originally defined for solids and its extension to liquids has been questioned[8]. It can be seen from equation (8) that estimates of the adiabatic gradient are further hampered by uncertainties in the variation of c_p (or γ). When values of the various parameters are assigned, the adiabatic gradient in the core may be estimated once a temperature has been 'fixed' at any point.

Write

$$X = \frac{\text{Excess temperature gradient}}{\text{Adiabatic temperature gradient}} = \frac{\beta c_p}{\alpha^2 \varphi T} = \frac{\beta}{\alpha\gamma T} \tag{9}$$

Taking extreme values of the parameters ($T = 5{,}000$ K, $\alpha = 10^{-5}$ K^{-1}, $\gamma = 1.75$), the minimum value of $X \simeq 11\beta$. Hence even if $|\beta|$ is as small as 0.1, the excess temperature gradient is equal to the adiabatic. It would thus seem that $|\beta|$ is unlikely to exceed ~0.01. This is a significant constraint on any future study of core dynamics. Dziewonski *et al.*[9] independently concluded that densities and velocities in the core (and lower mantle) are consistent with the Adams–Williamson equation (β = 0) to < 0.2%. It is not yet certain whether additional constraints on β will allow us to determine the gross stability structure of the outer core. In the meantime, it is interesting to examine the properties of some models of the Earth that have been proposed. For Model PEM-A, β > 0 throughout the core, thus permitting convection. For other models (1066A, 1066B) β is positive over most of the outer core, although becoming negative over ~ the middle 700 km Values of β are so large for these models that the corresponding values of τ lead to highly super-adiabatic gradients near both boundaries of the outer core.

If the outer core does have regions of instability adjacent to its boundaries with the mantle and inner core, separated by a stable central region, it is interesting to speculate whether convection in either of the unstable regions could power the geodynamo Verosub[10] recently suggested that the Earth's magnetic field was the sum of two oppositely directed dipoles and was able to account for reversals of the field without requiring a self-reversing dynamo. He suggested that the two sources are located in the inner and outer cores. The source field in the liquid outer core could be generated by magnetohydrodynamic action, but it is much more difficult to postulate a source mechanism in the solid, inner core. It is perhaps possible that at the pressures and temperatures existing in the inner core, new solid state phenomena exist which could lead to permanent magnetisation On the other hand, it is possible that both sources are in the outer core in two different unstable regions as indicated above Such a possibility would also be compatible with Kono's[11] model of the Earth's magnetic field in which he postulated ten dipoles in the core with equal moments and directions either parallel or anti-parallel to the rotation axis. It is interesting to note that Gubbins (unpublished) has shown that, contrary to expectation, the absence of convection, or at least sufficiently vigorous convection, in the outer regions of the core does not inhibit the generation of magnetic field. Specifically, he has shown that circulation on a global scale surrounded by a stable, electrically conducting region is more efficient for magnetic field generation than fluid motion throughout the entire outer core.

It must be emphasised that the arguments given in this note have assumed a homogeneous core of one component only. Thus, in addition to the entropy term in equation (5) for the density gradient we must add terms for additional light alloying components such as sulphur or silicon to obtain a more realistic model of the Earth's core. The relative importance of non-adiabatic conditions and inhomogeneities are being investigated.

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The 20-yr oscillation in eastern North American temperature records

IT has been argued (see for example ref. 1) that there is little or no reliable evidence for 11- or 22-yr cycles in meteorological time series, and that even if a significant spectral peak exists, the identified cycle may not maintain its phase and amplitude sufficiently to allow reliable prediction. To investigate this we have examined January temperature records for 19 stations in eastern North America. Our results show that: first, a pervasive 20-yr peak exists in the spectra of January temperatures in eastern Canada and the United States; secondly, the 20-yr oscillation was largely in phase over this region until approximately 1960; and thirdly, the predictability of this oscillation is, at best, only marginal.

With regard to spectral analysis the major problem of modern instrumental data is their availability only over times short by comparison with some of the interesting low frequency components. Maximum entropy spectral analysis (MESA) and filtering procedures now make possible, however, much more precise and reliable analysis. Recently, for example, MESA has provided convincing evidence of a 10.5-yr peak in the spectra of annual temperatures for selected North American stations, although no analysis of predictability was made[2].

For each record examined here, the Burg algorithm (Burg, unpublished, but see refs 3 and 4 for computational procedures) was used to estimate a 31-lag autocorrelation function from which the ME spectrum was calculated. To examine the pre-

Table 1 Stations used in this study. For ease of comparison all variances of bandpass filtered data have been normalised to percentage of observed variance

Station	Years of record	Observed variance (°C)^2	Bandpass variance (%)	75% Lower limit bandpass variance (%)*
1 Sable Island	1898–1974	2.09	14.5	10.9
2 Charlottetown	1889–1974	5.28	8.7	6.5
3 Eastport	1874–1975	4.76	10.8	8.2
4 Montreal	1875–1974	7.09	9.9	7.5
5 Hanover	1835–1975	7.69	9.7	7.3
6 Toronto	1841–1974	7.19	7.1	5.4†
7 Blue Hill	1832–1975	5.82	10.2	7.7
8 New Haven	1779–1975	5.17	8.5	6.4
9 New York	1869–1975	6.08	12.5	9.4
10 Pittsburgh	1875–1975	7.98	7.4	5.6†
11 Columbus	1879–1975	9.19	7.7	5.8†
12 Washington	1873–1975	6.74	12.3	9.3
13 Lynchburg	1872–1975	6.09	8.2	6.2†
14 Cape Hatteras	1875–1975	6.37	12.6	9.5
15 Charleston	1823–1975	6.04	11.9	9.0
16 Columbia	1888–1975	6.23	12.5	9.5
17 Mobile	1873–1975	6.55	13.3	10.0
18 Memphis	1872–1975	7.58	8.6	8.6
19 Nashville	1873–1975	7.88	9.1	6.9

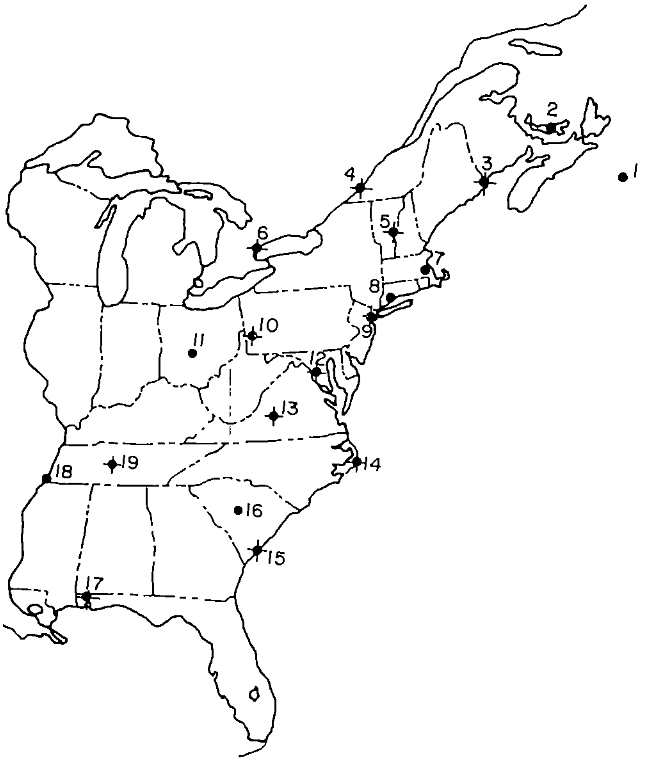
*Expected variance bandpassed: 6.3% (white noise).
†Overlaps white noise range.

dictability of the meteorological records we used the ME optimal filtering approach[4,5]. The computational procedure consists essentially of characterising a time series by an autoregressive process, the basic idea being that ME supplies a unique way of choosing unknown autocorrelation values to infinite lag (given a finite autocorrelation). These are chosen such that no information or entropy is added

$$\frac{\partial h}{\partial \varphi(k)} = 0 \quad |k| \geqq N+1$$

where h is entropy, $\varphi(k)$ is the autocorrelation at lag k, and N is the maximum lag number. Consequently, the optimal prediction filter for arbitrary distance may be determined by MESA. Since records can be extended into the past and future, filtering can be accomplished without loss of endpoints in the real data domain.

Fig. 1 Location of the 19 stations used in this study. Numbers refer to Table 1. Stations shown with + used to form regional temperature time series.



We used the 19 stations shown in Table 1 and Fig. 1. All data were taken from the World Weather Records supplemented by data published by the Canadian and US Weather Services. For one of the stations (Hanover, New Hampshire) we calculated the ME spectra of each of the four seasons and of each month. A 20-yr peak was prominent only in the winter spectrum, and, of the winter months, only in January. It is perhaps significant that this is when the Northern Hemisphere

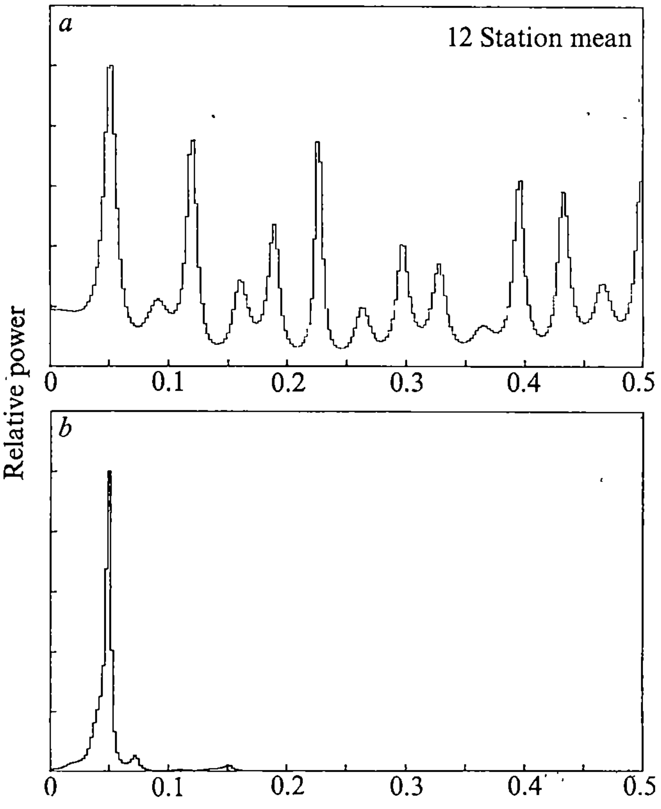


Fig. 2 *a*, Maximum entropy spectrum of regional January temperature time series. The 12 stations indicated + in Fig. 1 were used for the time period 1875–1974. *b*, Maximum entropy spectrum of double solar cycle time series.

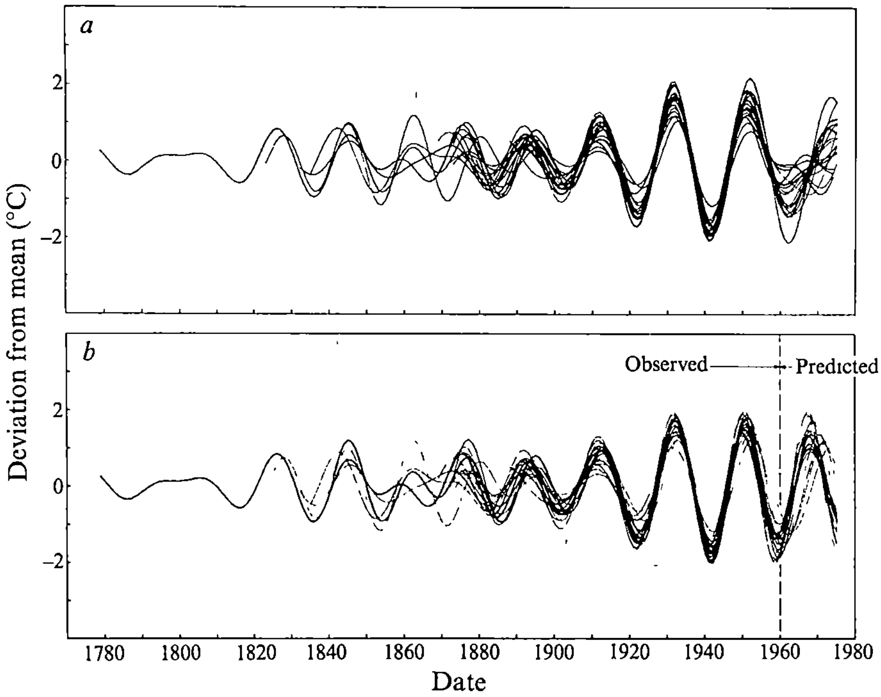


Fig. 3 *a*, Bandpass filtered January temperatures. *b*, Same as *a* but shows optimal prediction 1960–75.

circulation is most vigorous and the northward transfer of heat by large scale eddy activity is at its maximum[6].

In addition to calculating the ME spectrum for each record we performed the following operations on this set of stations: (1) Formed a regional January temperature time series using the 12 stations indicated in Fig. 1 for the period 1875–1974 and calculated the ME spectrum of this regional series (Fig. 2*a*). (2) Optimally extended each record and then isolated the 20-yr oscillation by bandpass filtering (Fig. 3*a*). (3) Truncated the data at 1959 and then performed the same optimal extension and filtering as shown in Fig. 3*b*.

The salient feature of each of the individual ME spectra of January temperatures was the prominent peak at ~ 20 yr. The spectra of the regional temperature series (Fig. 2) indicate that the 20-yr oscillation has been substantially in phase over the region. The finite width of the peak, however, is indicative of some drifting of phase and variation in amplitude—what we term a stochastic rather than a deterministic process.

Figure 2*b* shows the ME spectrum of the double solar cycle calculated from data given by Chernosky and Jose[7,8]. The coincidence of the 20-yr peak in this spectrum with the 20-yr peak of the temperatures is striking. We emphasise, however, that this coincidence does not imply that correlation exists between the two phenomena, let alone any causality, but to attribute this coincidence solely to chance seems quite unjustified.

Figure 3*a* shows the bandpass filtered records for all 19 stations. The relevant points are: (1) the near synchronism and stability of phase relationships from about 1890 until the late 1950s, and (2) their absence before 1890 and after 1960. Figure 3*b* illustrates the danger of simple projection as a means of prediction. Although there is some phase drift in the predicted records, the chaotic behaviour of the data would have obviated any regional prediction.

To provide a statistical measure of the significance of the spectral components isolated by the bandpass filter, we calculated their variance and the lower 75% confidence limit using the χ^2 distribution for comparison with the expected variance of bandpassed white noise in this same frequency range. In only four instances did this limit include the expected value of white noise.

In conclusion we feel the results presented here demonstrate a pervasive 20-yr oscillation in January mean temperatures over a substantial portion of eastern North America. This, coupled with Currie's[2] demonstration of a 10.5-yr spectral peak, illustrates the resolution inherent in MESA when dealing with the generally short time scales of instrumental meteorological data, a resolving power generally unavailable at the time of Monin and Vulis's[1] review.

In addition, optimal extension and bandpass filtering have shown that the sample stations were oscillating substantially in phase from about 1890 to about 1960. Comparison of the optimally extended data from 1960 on with the actual data clearly demonstrates the inherent danger of predicting quasi-periodic phenomena on the basis of simple projection.

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The hydration of Portland Cement

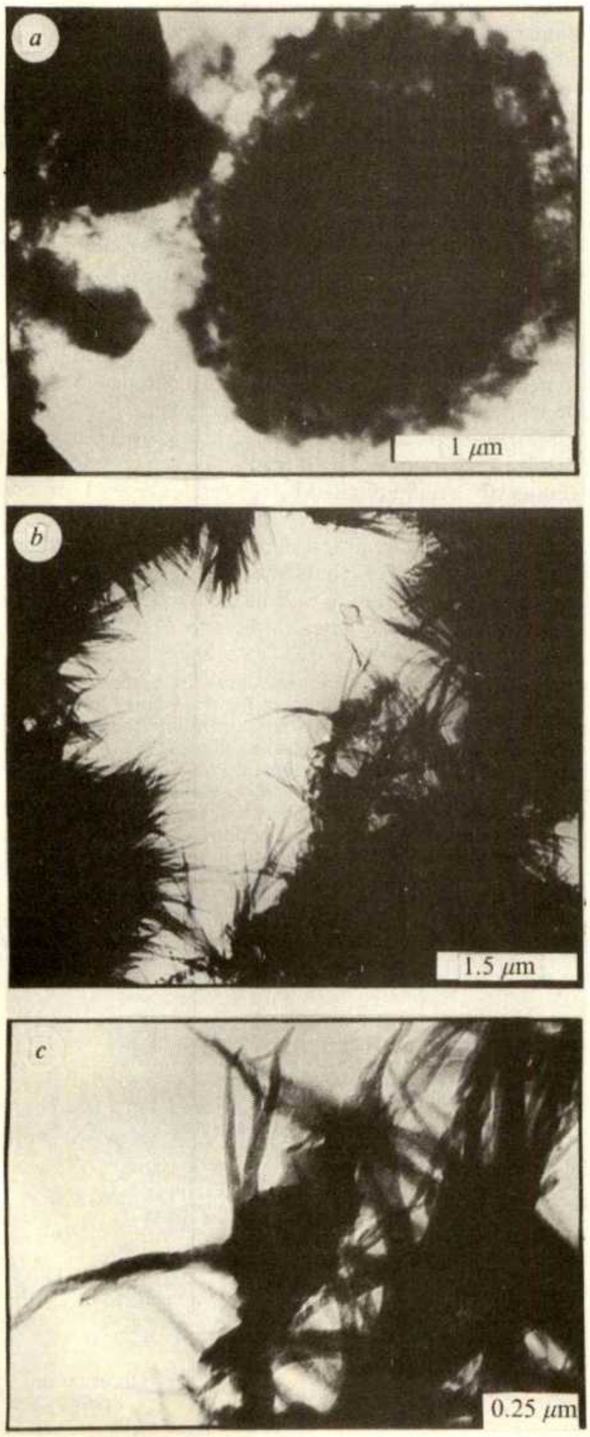
ALTHOUGH extensive X-ray data have been recorded, until relatively recently[1] the microstructural changes which attend the hydration of Portland Cement have received scant attention. We here report electron transmission observations of the reaction and draw attention to analogous silicate models which suggest a probable mechanism.

Scanning electron microscopy of cement specimens which have been dried for various periods after addition of water to di- or tri-calcium silicate mixtures (Portland Cement) show that there are two stages in the reaction involving (1) the formation of an initial coating of gelatinous hydrate (conventionally referred to as calcium silicate hydrate gel) on the anhydrous crystals, and (2) subsequent growth from this coating of fine fibrillar material, the interlocking of which is thought to cause the cement to harden and develop a high compressive strength.

This picture is compatible with the results of calorimetric studies which show that the rates of heat evolution over periods of 10^2–10^3 h can be interpreted as resulting from a diffusion-controlled phase reaction proceeding by nucleation and growth.

Studies have been made in this laboratory by direct observations in transmission within a 'wet' environmental cell (2) placed in a 1-MeV electron microscope. Time lapse pictures, Fig. 1, confirm the above mentioned two-stage nature of the reaction, but also provide further detail of the fibrillar product, suggesting (Fig. 1*c*) that it is not solid crystalline material but is irregular and tubular in form. The product is not crystalline in a conventional sense and does not yield coherent diffraction patterns, and the driving forces which might lead to such growth, following an earlier incubation period might seem to be obscure.

Fig. 1 Cement and water samples viewed in the environmental stage of the HVEM. *a*, after 3 h, showing the initial gel coating around a cement grain and small angular crystals of Portlandite (left). *b*, after 1 d, showing fibrillar development of C–S–H gel around the cement grains, illustrated in detail in *c*.



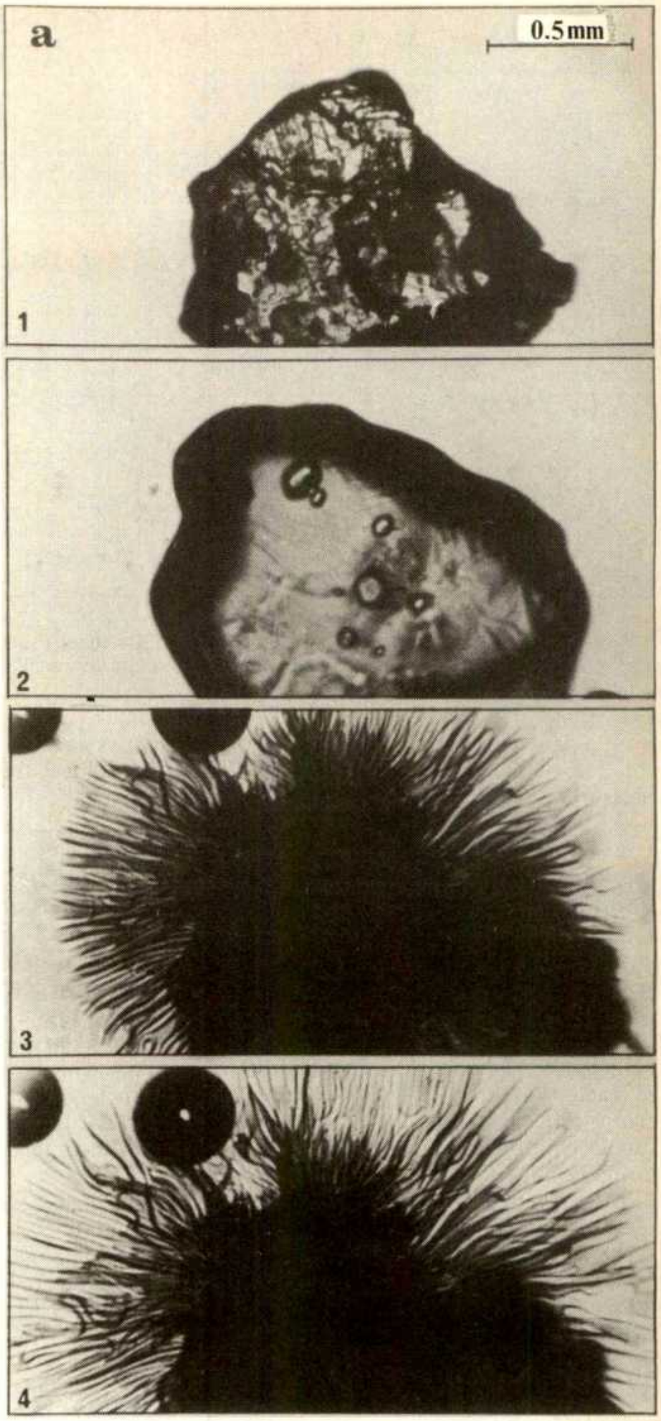


Fig. 2 Sequence showing the development of a 'silicate garden' from a crystal of $Co(NO_3)_2$ immersed in dilute sodium silicate solution. The time interval (1–4) is ~30 s. Optical micrograph.

The growth of hollow or tubular forms is, however, not a unique phenomenon, being characteristic of a wide range of compounds which occur in so-called 'silica gardens'. Figures 2*a*–*d* are time lapse optical micrographs of an aqueously soluble salt ($Co(NO_3)_2$) which has been brought into contact with sodium silicate solution, and although the rates of formation and scale are some 10^2 or 10^3 greater than those illustrated in Fig. 1—for Portland Cement—the striking analogy cannot be overlooked.

The mechanism by which the tubular 'silica gardens' form involves osmosis through the initial gelatinous coating which acts as a semi-permeable membrane, so that with continued

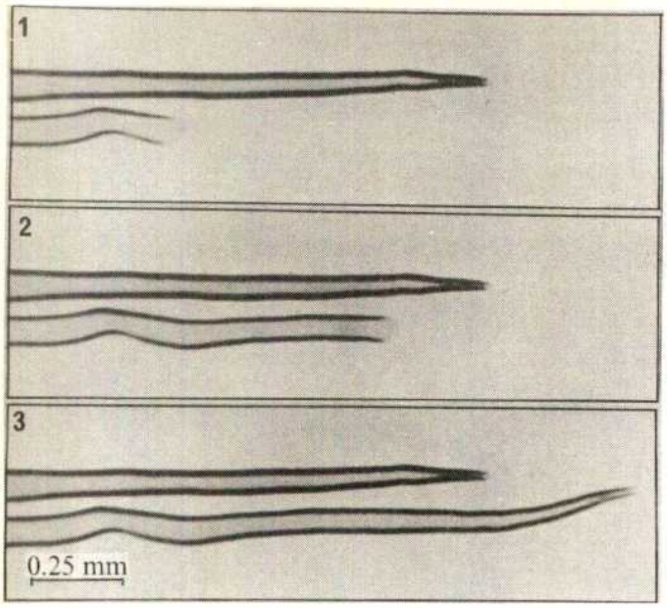


Fig. 3 10-s time lapse sequence of two cobalt silicate tubes (as in Fig. 2) illustrating in detail the tip reaction cone and terminal tapering effect. Optical micrographs.

solution of the salt crystal within the primary envelope, the pressure rises until rupture occurs locally. The jet of salt solution which errupts into the silicate solution precipitates continuously a tube of solid material, and as salt solution is pumped along the tube so formed—from the primary envelope—almost steady-state growth can proceed for considerable distances. Details of the growth (Fig. 3) show how a cone of salt solution reacts with silicate solution and builds up a solid wall until viscous drag and falling pressure limits the flow and leads to necking down or closing of the tubes. These silicate tubes are of variable section, are remarkably strong and brittle when dry, but do not yield coherent X-ray diffraction patterns. This type of reaction occurs with water soluble salts of any metal cations except those in Group 1A of the Periodic table, and further, should be possible whenever the initial reaction product is an insoluble but semi-permeable material.

We suggest that the analogy between the 'silica garden' reaction and that which occurs during the hydration of Portland Cement is close, and goes some way towards explaining why cement fibrils are not faceted solid crystals such as those commonly found during crystal growth from aqueous solution. The two-stage character of the reaction is then explicable in terms of a model for which osmosis provides the driving force for secondary growth, and although direct extension to include cement reaction products must involve a different chemical combination, the diffusion species in each case would be water and the initial coating a colloidal silicate gel.

The above outline necessarily raises many points of detail and speculation which require a more complete and discursive account, to be published elsewhere. Further work is in progress in this laboratory, the better to establish the tubular shape of Portland Cement hydration products, and to quantify the factors which control the growth of silica gardens, with the intention of confirming, or otherwise, the connection between these materials and the mechanism by which they grow. It is worth noting that silicate membranes are indeed semi-permeable (to water) and that the lengths and diameters of the tubes in any one system (for example $CaCl_2$, $Co(NO_3)_2$, $FeSO_4$, with sodium silicate) decrease with reaction rate, so that extrapolation to the slower growing and finer cement hydrates is both in the correct direction and corresponding scale.

Establishment of the reaction mechanism is a necessary step if this important process, of cement hardening, is to be understood and controlled.

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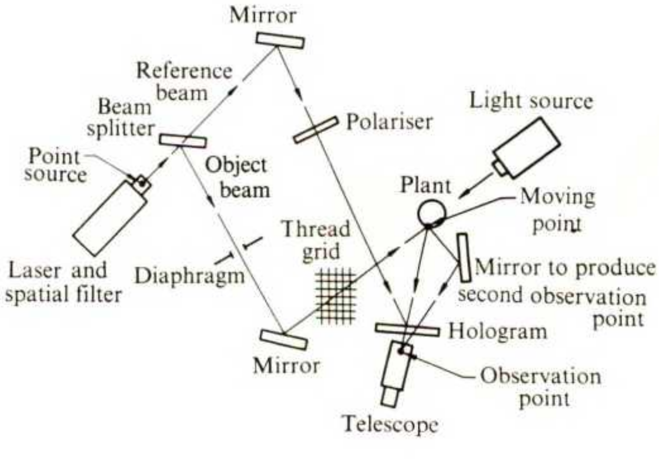
Analysis of transient plant movements by holographic interferometry

Holographic interferometry is a photographic technique which can be used to measure minute displacements of objects[1]. Williams[2] has shown holographic interferograms of painted wheat shoots, but in his initial work there was no attempt to make quantitative measurements of either plant displacement or, more importantly, transient plant response. The present work demonstrates the potential of holographic interferometry in plant movement analysis by showing previously unobtainable data on the bending and short term transient response of a mature, slow moving plant. Our results suggest that the holographic interferometric technique may provide the means to measure accurately such important plant movement parameters as velocity and displacement with much higher resolution than is available from any other method. In addition, the technique substantially improves the time resolution of plant movement analysis, thus enabling more detailed observation of the tropic response as a function of time.

A hologram is a photographic recording of the interference pattern between an object light field and a reference field. When the hologram is reilluminated by the reference field, diffraction causes the object field to be reconstructed. A double exposure hologram or holographic interferogram[3] is formed when two holograms are taken on the same photographic plate. When a double exposure hologram is visualised, the two reconstructed images interfere as if they coexist in space. The displacement, *d*,

Fig. 1 The holographic arrangement consisted of a He–Ne laser with spatial filter, a beam splitter for producing the reference and object beams, two mirrors for controlling the beam directions, a polariser for controlling the reference beam intensity, a diaphragm for eliminating extraneous reflections, and a holographic camera. Additional elements useful for plant movement studies were a thread grid which cast a shadow grid on the plant, one to three mirrors for obtaining additional observation points, a telescope mounted on a sextant for measuring angles and counting fringes, and a monochromator for producing the phototropic stimuli. The components were positioned on a 2.5 × 61 × 100-cm steel plate using magnetic mounts. Vibration isolation was obtained by supporting the apparatus on four inflated rubber inner tubes.



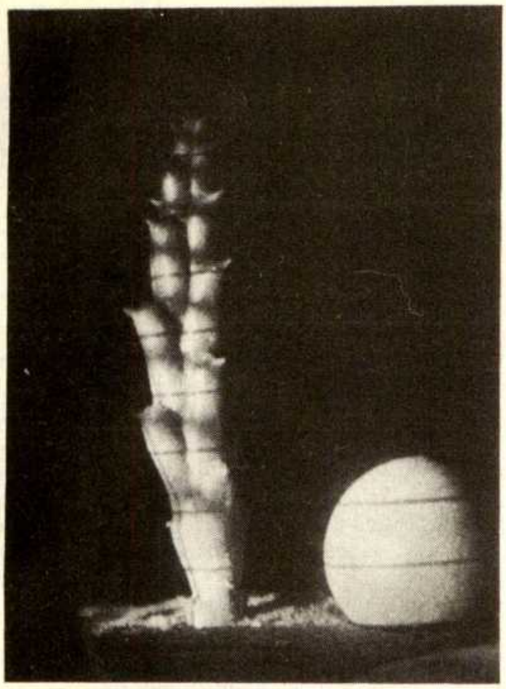


Fig. 2 Holographic interferogram of *S. variegata* Linn showing horizontal bands indicating motion of plant between exposures. Photographed through the hologram.

of a point on the object can then be calculated by evaluating the vector expression

$$N = \frac{1}{\lambda}(\mathbf{k}_1 - \mathbf{k}_2)d \qquad (1)$$

where N is the number of fringes counted from a stable reference point at the base of the plant to the point of interest, $\mathbf{k}_1$ and $\mathbf{k}_2$ are unit vectors in the source and observation directions respectively, d is the displacement vector, and λ is the wavelength of the radiation used to make the hologram.

The experimental apparatus used in the present study is illustrated in Fig. 1. The room temperature was controlled, and precautions were taken to eliminate air currents and extraneous light. *Stapelia variegata* Linn was used because it is easy to maintain in the laboratory, and its size and smooth contour result in easily discernable fringes.

To capture transient plant responses, a sequence of holographic interferograms was taken using an automatic camera loaded with a strip of Kodak SO243 35-mm holographic film. It was necessary to allow the camera to equilibrate for 1 min between advances of the film so that the new frame would stabilise thermally and mechanically. Thus the resultant sequence of double exposure holograms represents the displacements during 4-min intervals taken every 5 min. The reconstructed image of a typical holographic interferogram is shown in Fig. 2.

In the present set of experiments, the entire side of the plant was illuminated with 0.02 mW cm^{-2} 450 nm light obtained from a monochromator. This blue light was chosen because it falls in the most active area of the phototropic action spectrum of typical plants. The procedure was to wait until the plant had reached thermal and phototropic equilibrium and then suddenly expose it to a constant phototropic stimulus. The time intervals between exposures were measured to within 0.5 s using a Gralab timer.

Two main types of results were obtained from the experimental data—plots of plant displacement normal to the main axis of the plant body against height on the plant, and plots of displacement and velocity as a function of time of a particular point on the plant. Figure 3 is a typical example of the first type of result. From this data, the precise bending of the plant in response to phototropic stimuli can be studied. Figure 4 illustrates the second major result. Displacement and velocity of a point 3.5 cm from the base of a 4.2-cm high plant are plotted over a 75-min timespan. The increase in velocity on stimulus application can be shown to correlate well to a function of the form $1-e^{-\alpha t}$, the decay on removal of the stimulus to $e^{-\alpha t}$. The solid lines in Fig. 3 are based on this assumption with $\alpha = 0.2$.

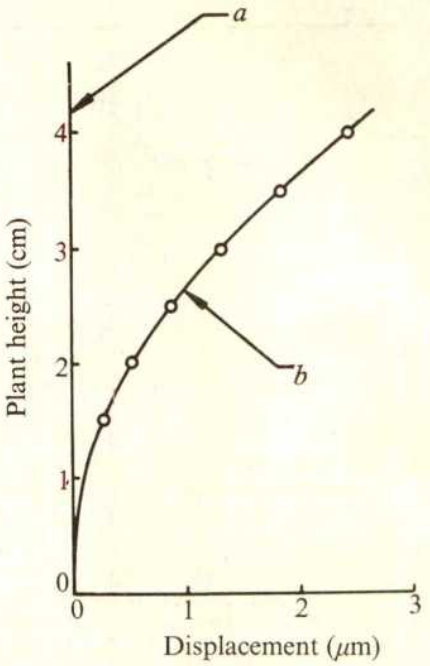


Fig. 3 Quantitive plot of the bending undergone by the plant shown in Fig. 2. The fringes represent the movement of the plant in response to 0.02 mW^{-2} cm 450 nm blue light over a 4-min time interval, after it had been exposed to 20 min of 0.02 mW^{-2}cm 450 nm blue light. *a*, plant position at time of first exposure; *b*, at time of second exposure (4 min later).

Conventional techniques for the measurement of plant motions have various limitations and shortcomings. The direct measurement of displacements by such methods as shadowgraphs and time-lapse photography has very low resolution and is therefore restricted to relatively large movements. Systems making use of mechanical displacement detectors require physical contact with the plant. Techniques using microscopes can examine only small areas on the plant at a time.

Holographic interferometry is useful for measurement of displacements in the micrometric range which can be expected to occur a few minutes to an hour after application of a stimulus. Since different mechanisms may be involved in the long term movements which have been observed with coleoptiles, holographic interferometry opens up a completely new area of investigation. Each hologram contains an image of the entire plant so that it is possible to quantify the changes with time of displacement, velocity and acceleration at any point on the plant visible from the observation point. Furthermore, the high sensitivity makes it feasible to run phototropism studies on mature plants for the first time. Holographic interferometry also provides a permanent, highly accurate record of plant movements. Due to the large amount of data contained on a single hologram, computerised analysis may be useful to obtain detailed displacement, velocity and acceleration maps over the entire plant.

This is the first time dynamic plant movement data involving such small displacements has been obtained over such a short

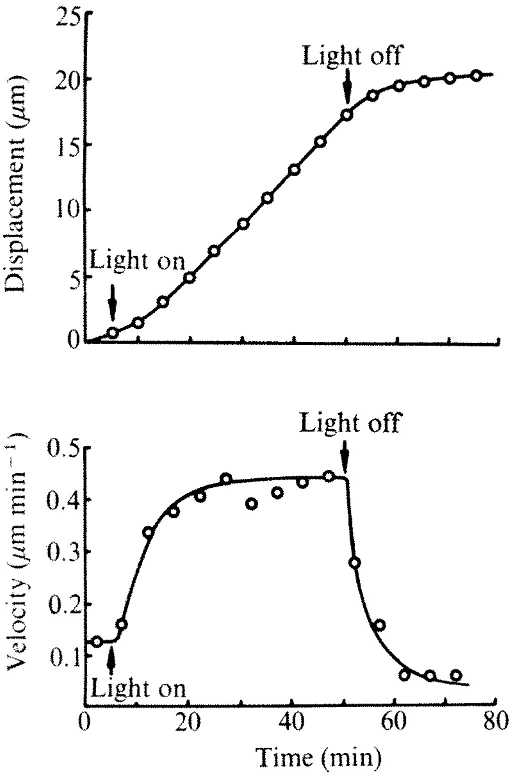


Fig. 4 Phototropic displacement undergone by a point 3.5 cm from the base of a 4.2-cm high plant in response to 0.02 mW cm^{-2}, 450 nm blue light from a monochromator. Before $t = 5$ min the stapelia was undergoing a small constant motion in darkness. The blue light stimulus was applied at $t = 5$ min and removed at $t = 50$ min. *a*, Total tip displacement; *b*, tip velocity. The points are experimentally determined, whereas the lines represent the best fit assuming the velocity data are of the form $1 - e^{-0.2t}$ on stimulus application and $e^{-0.2t}$ on stimulus removal.

time frame. Thus, the data are of interest simply to their unique nature. In addition, the results indicate an unexpectedly rapid response to phototropic stimuli by what might be considered a sluggish plant. Perhaps the most important observation is the unexpected conformity of the velocity data to an exponential function suggesting the applicability of a second order linear differential equation to plant movement analysis. Overall, the results obtained thus far suggest that the application of holographic interferometry to plant movement analysis will make possible more complete understanding of the mechanisms of plant movement.

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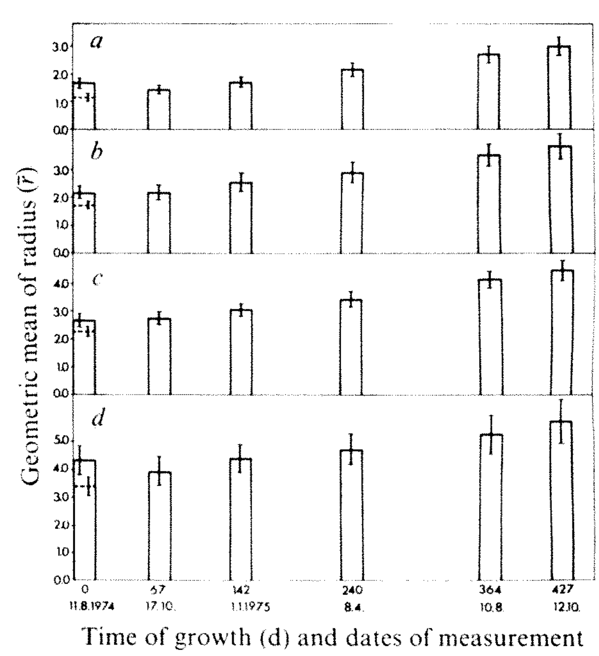
Skeletal regeneration in a Red Sea scleractinian coral population

Corals may be harmed by their natural enemies or by environmental conditions. The ability of scleractinian corals to regenerate damaged parts has been documented qualitatively by many investigators[1–10], but detailed quantitative data on the rate of regeneration have not been reported. Most reports dealing with the destruction of coral reefs have emphasised the long time required for recovery[11]. I have found, however, that the rate of skeletal regeneration in a population of the branched coral *Stylophora pistillata* (Esper) is surprisingly fast. During the first 2 months of regeneration, damaged colonies grew twice as fast as intact control colonies. Within the same colony, damaged branches grew faster than intact branches, which resulted in a tendency to regain symmetry lost through breakage. Larger colonies showed a better capacity to resist damage than smaller colonies.

Stylophora pistillata is one of the most important scleractinian corals in the Gulf of Eilat[12] because of its abundance and major contribution to the reef framework[13]. Seventy colonies of *S. pistillata*, growing in shallow water (3–4 m deep) at Eilat, were damaged by breaking some of their branches with a diver's knife. All damaged colonies were numbered by means of plastic tags and photographed before and after breakage, as well as during recovery. The growth rate of approximately 200 intact colonies studied previously in the same area[14] served as a control for comparative analysis. The study lasted from August 11 1974 to October 12 1975. The length, width and height of each colony were measured under water every 2–3 months. Length is defined as the distance across a coral between the tips of branches which are farthest apart; width is a measure perpendicular to the length axis; height is orthogonal to the width and length axes. Since the shape of *S. pistillata* approximates a sphere, the colonies were divided into size groups, according to the geometric mean of their radius, $\bar{r}$ (Fig. 1).

The initial response of the coral to breakage was to cover the exposed areas rapidly with living tissue to prevent settlement of fouling organisms. Fishelson[8] found that after a catastrophic low tide that caused partial desiccation or death of many colonies of Red Sea scleractinian corals, regeneration in

Fig. 1 Skeletal growth rate in damaged colonies of *S. pistillata*. The corals were divided into size groups according to their geometric mean radius ($\bar{r}$) after breakage. ($\bar{r} = (l \times w \times h)^{1/3}/2$ where l is length, w is width, and h is height (cm).) The $\bar{r}$ of each size group is given (in cm) by the height of the bars. The vertical lines in the middle of the bars represent the standard deviations. The horizontal broken line in the left bar of each size group represent $\bar{r}$ after breakage. The percentage decrease in $\bar{r}$ after breakage for each size group was 29.08, 19.46, 14.20 and 21.74 in order of smallest to largest size group. The estimated number of days of growth (± s.d.) required to regain original $\bar{r}$ were 122±17, 67±7, 51±5 and 116±15 in order of smallest to largest size group. The expected number of days of growth required to regain original $\bar{r}$ after 25% breakage are 104±14, 86±10, 90±9 and 133±17 in order of smallest to largest size category. *N*, number of colonies measured. *a*, $N = 11$, $\bar{r} = 1.00$–1.50; *b*, $N = 14$, $\bar{r} = 1.51$–2.00; *c*, $N = 16$, $\bar{r} = 2.01$–3.00; *d*, $N = 10$, $\bar{r} = 3.01$–4.00.



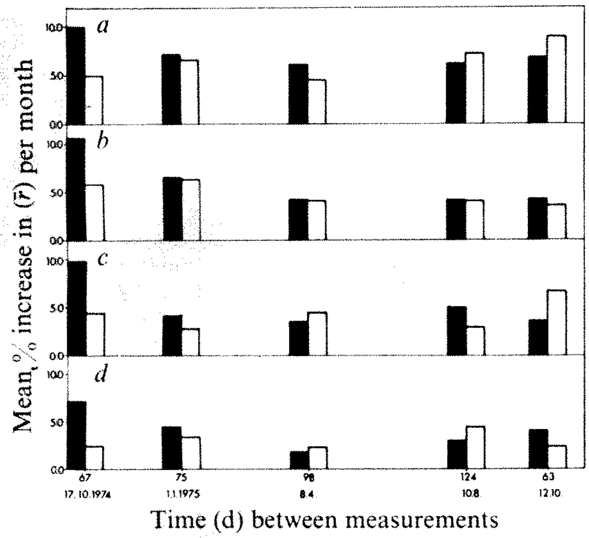


Fig. 2 Comparison between the mean percentage increase in geometric mean of radius ($\bar{r}$) per month ($\Delta\bar{r}$ %/month) of damaged corals (black bars) and intact control corals, belonging to the same size interval (blank bars). Average values of $\bar{r}$, in the different dates of measurements are given in Fig. 1.

$$\Delta\bar{r}\ \%/\text{month} = \frac{30}{\Delta t}\,\frac{(\bar{r}_t - \bar{r}_{t-1})\times 100}{\bar{r}_{t-1}}$$

where Δt is time (d) between measurements.

N_1, Number of colonies measured in regenerating groups; N_2, number of colonies measured in intact control groups. a, $\bar{r} = 1.00$–1.50, $N_1 = 11$, $N_2 = 23$; b, $\bar{r} = 1.51$–2.00, $N_1 = 14$, $N_2 = 19$; c, $\bar{r} = 2.01$–3.00, $N_1 = 16$, $N_2 = 17$; d, $\bar{r} = 3.01$–4.00, $N_1 = 10$, $N_2 = 9$.

branched colonies started with reorganisation and reactivation of the peripheral soft tissue along the line of destruction. The regenerated tissue grew over fouling algae, which had settled on the dead coral skeleton, resulting in a "sandwich structure", in which the old skeleton and newly deposited skeleton engulfed the algae. According to Connell[15], if tissue damage on a coral surface is sufficiently large, recovery may not be rapid enough to exclude colonisation by fouling organisms, that may ultimately kill the coral. Fifty-one out of seventy damaged colonies survived throughout the study (Fig. 1). Most of the smallest colonies ($\bar{r} < 1.00$ cm), however, were covered by filamentous algae within a few days of being broken, and shortly afterwards they were dead. This confirms Connell's[15] conclusions that larger colonies have a better capacity to resist invasion and damage than smaller ones of the same species.

Figure 1 presents the extent of skeletal damage caused to each size category and the rate of regeneration and growth. The percentage decrease in $\bar{r}$ of different size groups varied from 14% to 29%. Assuming linear growth between the periods when measurements were taken, and taking into account the different standard deviations of $\bar{r}$, within each size category (Fig. 1), I estimated the number of days of growth required by the damaged corals to attain their size before breakage (see legend to Fig. 1). To compare the different size groups with respect to their capacity for skeletal regeneration, the expected average number of days of growth to regain original size after 25% breakage was calculated for each group (see legend to Fig. 1). The largest size group ($\bar{r} = 3.01$–4.00 cm) required a significantly longer time to attain its original size than did any of the other groups (t tests, $P < 0.05$), which strengthens the assertion that this species grows more slowly as it ages[14].

Figure 2 compares the mean percentage increases in $\bar{r}$ per month ($\Delta\bar{r}$ %/month) in the damaged corals and the intact control groups. During the first 2 months of regeneration, all damaged corals grew twice as fast as the intact control corals. After the damaged colonies had regained their original size, no significant differences were observed between the average growth rate of damaged and intact colonies (t tests, $P > 0.05$). In general, the growth of both regenerating and intact colonies slowed down during the winter (December to April).

Ninety per cent of the population exhibited accelerated growth at the damaged portions of the colony (compared with intact branches) as well as a tendency to regain symmetry lost by breakage. Similar observations have been reported before[3–6]. This phenomenon suggests some integrated response between the parts of a single colony[15]: individual polyps act synergistically to differentiate tissue and create edge zones of proliferation[16], and locally to accelerate skeletogenesis. The idea of integrated response is strengthened by evidence of transfer of energy-rich organic products between adjacent parts of a colony[17]. Thus, Pearse and Muscatine[17] suggested that translocated products of zooxanthellae may enhance calcification rates in corals, by serving either as specific substrates in the organic matrix or as general energy sources.

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Persistence in continuous light of a circadian rhythm in the mosquito *Culex pipiens fatigans* Wied.

Brady[1] has discussed Truman's[2] hypothesis that circadian clocks can be divided into two types: type 1 in which light seems to act directly on the oscillator, stopping it in constant light (LL); and type 2, in which the photoreceptor and oscillator are separate and the rhythm is free-running in LL. Typically, in insects, developmental events such as eclosion seem to be controlled by the first type, and daily behavioural activities by the second[2]. In Diptera, however, behavioural rhythms seem to be rapidly damped out by LL[1]; for example in the mosquito *Anopheles gambiae*, the 'clock' controlling flight activity seems to stop when the light phase is prolonged, and to restart at the next onset of darkness[3], thus showing type 1 characteristics. Previously, the only known exception among the Diptera was *Aedes aegypti*, a light-active mosquito, in which the flight activity rhythm persists, rather weakly, in LL[4]. Here I show that circadian flight activity in the mosquito *Culex pipiens fatigans* Wied. persists in LL, although in other respects

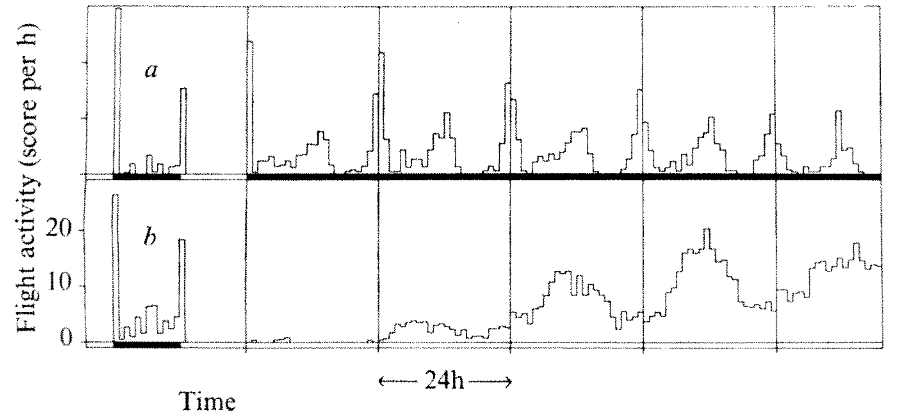


Fig. 1 Mean flight activity of individual female *C.p. fatigans*: effect of a change *a*, to DD ($n = 32$), *b*, to LL ($n = 46$).

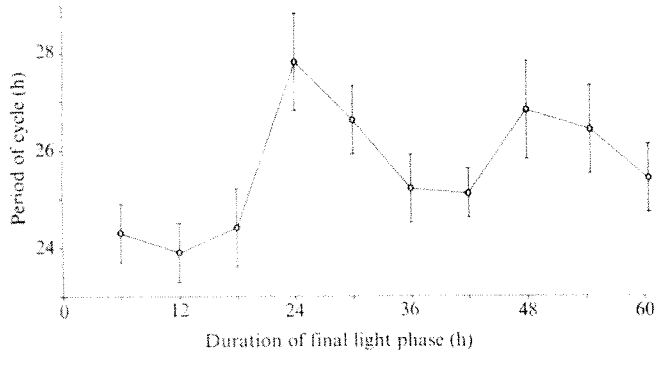
the rhythm is very similar to that in *A. gambiae*. As in *A. gambiae*[3] the period of the first cycle in constant darkness (DD) is distorted by the previous light period, but, in contrast, the magnitude of the effect depends on a rhythm that evidently persists in LL.

The culture of *C.p. fatigans* originated in the Gambia, West Africa; they were reared in alternating 12 h light: 12 h dark (LD 12:12) at 27 °C. The flight activity of individual females was monitored acoustically[5,6] (males gave almost identical results). The light intensity used in all experiments was 50 lx.

In LD 12:12, the pattern of flight activity in *C.p. fatigans* was very similar to that in *A. gambiae*[7] with peaks following light-off and light-on, and moderate activity during the dark phase. Cyclical activity continued in DD and, in contrast to *A. gambiae*, also in LL (Fig. 1). In DD the cycle was more strongly bimodal than in *A. gambiae*, with a secondary peak about 13 h after the main one. In LL, the cycle was unimodal with a period of about 26 h. Although activity was initially depressed in LL, it is possible to calculate that the first peak should have occurred about 18 h after light-on or 6 h after the normal time of light-off, when a small amount of activity did in fact occur (Fig. 1*b*, day 2).

When the change to DD was made after prolonging the final light phase, the rhythm was reset so that the first peak in DD always came in the first hour after light-off; shortening the final light period to 6 h did not reset the rhythm. In *A. gambiae*, light seems to have a delayed effect on the rhythm, causing a small but progressive increase in the period of the first cycle in DD, as the final light phase is prolonged[3]; but in *C.p. fatigans*, the effect seems to be much greater and the period of the first cycle in DD varied cyclically as the length of the final light phase was increased (Fig. 2). The period was greatest when light-off occurred about 6 h after a peak (or predicted peak) of activity in LL, and least when light-off occurred just before a peak. The response, therefore, seems to be determined by the phase of the rhythm in LL. In contrast, the lack of phase-determined responses in *A. gambiae* implies that there is no underlying undetected driving oscillation in LL.

Fig. 2 Effect on the period of the first cycle in DD of changing the duration of the final light phase.



For *C.p. fatigans* the period of subsequent cycles in DD was approximately 24 h in all cases, irrespective of the length of the previous light phase. Although the first peak in DD followed immediately after light-off, the phase of the free-running cycle was actually determined by the timing of the second peak, which depended both on the time of light-off and on the period of the first cycle.

Thus the mosquito flight-activity 'clock' seems to behave like one of Truman's type 2 clocks in *C.p. fatigans* and possibly also in the day-active *Ae. aegypti*, but like a type 1 clock in *A. gambiae* whose behaviour resembles that of the classical *Drosophila* eclosion clock[8]. Nevertheless, in *A. gambiae*, there is strong evidence[9] for the separation of this 'activity' clock from the 'developmental' clock controlling the time for pupation, whereas in *Drosophila*[10] the same clock seems to 'gate' both eclosion and adult behaviour. It seems unlikely that flight activity is controlled by fundamentally different types of clock in two species of mosquito with such similar basic patterns of activity. The differences between *C.p. fatigans* and *A. gambiae* could simply be due to different sensitivity thresholds, the oscillation persisting in LL (50 lx) in *C.p. fatigans* but not in *A. gambiae*; related evidence[3] suggests that in the latter it is damped out by 2 lx but not by 0.2 lx, although it can be entrained to a LD 12:12 regime at this intensity.

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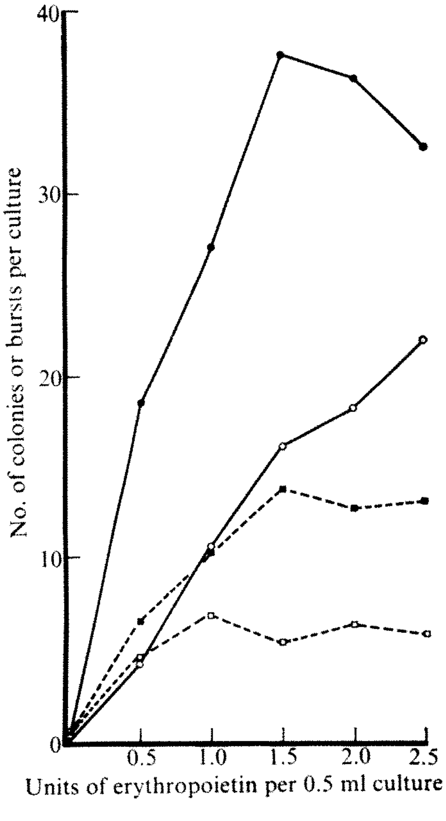
Induction of megakaryocyte colonies with platelet formation *in vitro*

COLONY culture methods utilising semi-solid media which facilitate extensive cellular differentiation *in vitro* have proved invaluable for the quantitative investigation of granulocytic and erythrocytic progenitors and the effector substances to which they respond[1,2]. Colonies of megakaryocytes have been produced from suspensions of mouse bone marrow or spleen cells in agar containing lymphocyte-conditioned medium[3]. But these megakaryocytes did not reach the stage of maturation at which significant platelet production could be demonstrated. We now report the development of megakaryocyte colonies with extensive platelet production in plasma cultures seeded with mouse bone marrow cells and containing either sheep or human erythropoietin in the medium. The proportional relation observed between the number of colonies produced and the number of bone marrow cells plated also provides the basis of a quantitative assay method for megakaryocyte progenitors in murine haemopoietic tissues.

Suspensions of mouse bone marrow cells were prepared from 8-week-old C57BL/6Ut mice and seeded at 1×10^5 cells in 0.5-ml volume plasma cultures prepared as described for the growth of erythropoietic bursts[2], except that beef embryo extract was replaced by an equal volume of medium NCTC 109 containing $CaCl_2$ to promote clot formation (final concentration of $CaCl_2$, 0.013 mg per 0.5 ml culture). Cultures were examined daily. A few megakaryocytes were seen on day 4 and by day 5 colonies were present. These increased in number and size up to day 7. The colonies consisted of two to 40 cells and appeared either as clusters of megakaryocytes or as megakaryocytes intermingled with the cells of an erythropoietic burst.

Fig. 1 Effect of variation in erythropoietin concentration on the number of megakaryocyte colonies and erythropoietic bursts produced in 0.5-ml plasma cultures seeded with 1×10^5 C57BL/6Ut bone marrow cells. Two preparations of erythropoietin were used: erythropoietin step III Lot. No. 3005 (Connaught Medical Research Laboratories, Willowdale, Ontario) prepared from anaemic sheep plasma (2.1 U per mg protein) and human urinary erythropoietin (pool H-11-TaLSL, 74.3 U per mg protein). Human erythropoietin was collected and concentrated by the Department of Physiology, University of the North-east, Corrientes, Argentina, further processed and assayed at the Hematology Research Laboratories, Children's Hospital of Los Angeles. ●, No. of erythropoietic bursts produced in cultures containing sheep plasma erythropoietin; ○, no. of erythropoietic bursts produced in cultures containing human urinary erythropoietin; ■, no. of megakaryocyte colonies produced in cultures containing sheep plasma erythropoietin; □, no. of megakaryocyte colonies produced in cultures containing human urinary erythropoietin.



When graded doses of sheep or human erythropoietin were added to cultures of 1×10^5 bone marrow cells, the numbers of both megakaryocyte colonies and of erythropoietic bursts at 7 d were found to be related to the concentration of erythropoietin added (Fig. 1). At an erythropoietin concentration of 1.5 U per 0.5 ml culture, the number of megakaryocyte colonies was proportional to the number of bone marrow cells plated (Fig. 2); about 1/4,000 bone marrow cells appeared to be the progenitor of a megakaryocyte colony.

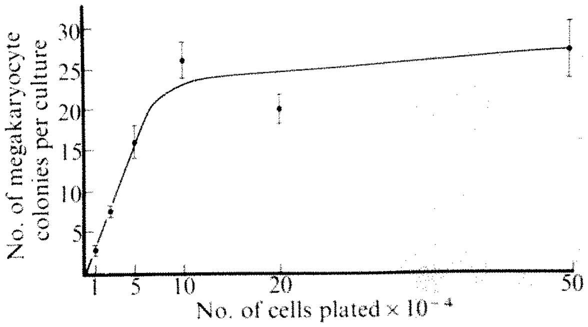


Fig. 2 Relationship between the number of mouse bone marrow cells plated and the number of megakaryocyte colonies produced after 7 d in plasma cultures containing 1.5 U of sheep erythropoietin per 0.5 ml of culture.

In preparations for light microscopy megakaryocytes were identified in the cultures by their morphology. They measured 20 to 100 μm wide and contained a single nucleus consisting of 2–16 lobes. The larger cells contained the more lobulated nuclei, presumably reflecting the higher degree of ploidy in these cells. By day 6 small rounded bodies 3–6 μm in diameter were seen at the periphery of many of these large cells. Because acetylcholinesterase has been used as a cytochemical marker for megakaryocytes and platelets, cells in some cultures were stained for this enzyme. Plasma cultures were fixed in 5% glutaraldehyde and treated by the method of Karnovsky and Roots[4]. The cells in the colonies and the small bodies at the periphery of the larger cells stained an intense brownish colour, indicating a high concentration of the marker enzyme.

Serial sections prepared for electron microscopy from 'pure' megakaryocyte colonies fixed and stained *in situ* showed large cells with multilobed nuclei, surface connecting tubules and demarcation channels in their cytoplasm, all characteristic of megakaryocytes. Small rounded bodies were present at the periphery of many of these cells, measured 5 μm or less in diameter and appeared either completely separate from the megakaryocyte cytoplasm or attached to the megakaryocyte along a partly formed demarcation channel in the cytoplasm. The fine structure of these, containing α granules, dense bodies, dense tubules and surface connecting tubules, was characteristic of platelets (Fig. 3).

In this study we have shown that megakaryocyte colonies can be grown in plasma cultures from their progenitor cells in bone marrow, and that maturation of these cells in the culture conditions described results in the production of platelets. In these experiments the effector substance responsible for inducing the production of megakaryocyte colonies was probably erythropoietin. Consistent with this interpretation are reports that certain pathological conditions in which erythropoietin levels are high are associated

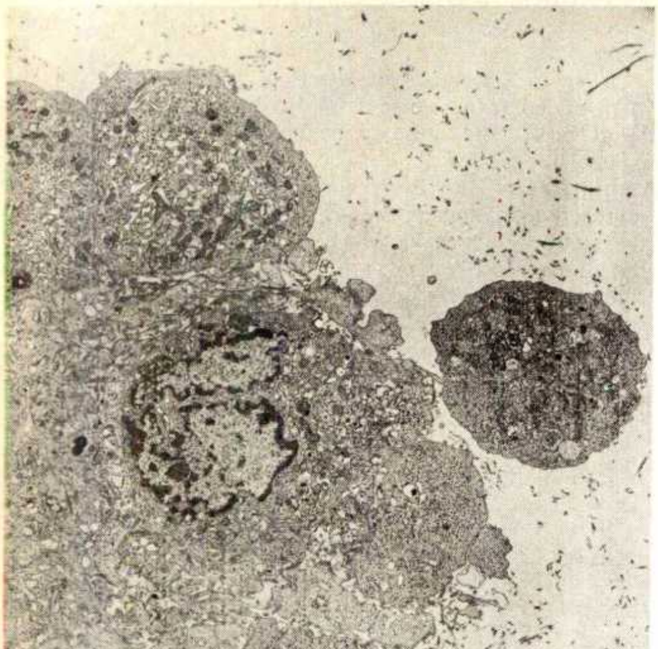


Fig. 3 Electron micrograph of a megakaryocyte from a colony that developed in plasma culture containing sheep erythropoietin, 8 d after it was seeded with C57BL/6Ut bone marrow cells. The culture was fixed in 3% glutaraldehyde, postfixed in osmic acid, flat-embedded in Epon, sectioned and examined in a Philips 300 electron microscope. Surface connecting tubules and demarcation channels are visible. The platelet at right was shown in serial sections to be separate from the cytoplasm of the megakaryocyte.

with thrombocytosis, and that correction of the primary pathological condition brings platelet levels to normal[5]. It is possible that a contaminant in the erythropoietin preparations was responsible for the effect, but we believe that this is unlikely. The megakaryocyte colony-stimulating factor would then have to be present as a contaminant both in the erythropoietin prepared from the serum of anaemic sheep, and in the erythropoietin prepared from the urine of anaemic patients. Furthermore, the concentration of the contaminant would have to be approximately the same relative to erythropoietin in both preparations (Fig. 2), although the erythropoietin concentrations of the two are vastly different (sheep erythropoietin, 2.1 U per mg protein; human erythropoietin, 74.3 U per mg protein).

It is interesting that Metcalf *et al.*[3] found that in cultures containing activated lymphocyte-conditioned medium, megakaryocyte colonies and granulocyte–macrophage colonies were produced, whereas in ours containing erythropoietin, megakaryocyte colonies and erythropoietic bursts were produced. The association of megakaryocyte colonies with granulocytic colonies on one hand and with erythropoietic bursts on the other raises the question as to whether the heterogeneity responsible for this phenomenon is at the level of the effector substance or of the responding progenitor cells.

The plasma culture system has already provided reliable assay systems *in vitro* for progenitors of erythrocytes and granulocytes; it should prove equally valuable for titrating megakaryocyte progenitors and for studying the mechanisms regulating thrombopoiesis.

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Susceptibility of Fanconi's anaemia fibroblasts to chromosome damage by carcinogens

INDIVIDUALS with certain genetic syndromes associated with chromosome damage show markedly increased incidence of cancer[1]. In one such syndrome, Fanconi's anaemia (FA), chromosomal breakage and rearrangement has been found in lymphocytes and fibroblasts[2,3] years before the development of malignant tumours. There is some evidence that cells from FA patients are especially sensitive to oncogenic agents. FA fibroblasts are abnormally susceptible to SV40 transformation[4], and lymphocytes from patients show increased chromosome aberrations after exposure to ionising radiation[5], or to alkylating agents[6,7]. In these reports, however, evaluation was based on cells which entered metaphase during brief and possibly toxic exposures to chemicals. We report here experiments in which FA fibroblasts were exposed to a direct-acting mutagen or carcinogen for a period of 6 d, ensuring chronic exposure of the cells during one or more cell cycles, until increased cell density inhibited further cell division. After subculture we could then assay chromosome damage in cells capable of entering a new cycle of cell division after removal from the chemical. We found that viable FA fibroblasts showed increases in chromosome aberrations after exposure to the mutagen or carcinogen at concentrations that had no effect in other cell strains tested. Since fibroblasts can be maintained in serial passage, their use in this protocol makes it possible to examine residual and possibly lasting effects of the treatment.

Human skin fibroblasts between passage 6 and 14 were used for all experiments. Fibroblasts from FA patients were originally obtained from Dr M. Swift (University of North Carolina at Chapel Hill), xeroderma pigmentosum (XP) fibroblasts were from the American Type Culture Collection (cell strain CRL 1158), and normal and trisomy 18 fibroblasts were derived from skin biopsies in this laboratory. Freshly distilled diepoxybutane (DEB) and ethylmethanesulphonate (EMS) were obtained through the courtesy of B. M. Goldschmidt (New York University Medical Center). Both are alkylating agents which require no metabolic activation *in vitro*. Mutagenic and carcinogenic activity have been demonstrated for DEB[8–11]; EMS is a well known mutagen which has been shown to be a weak carcinogen[12].

Preliminary studies were done to determine the toxicity of each compound. Cells were plated in 25-cm^2 flasks at a concentration of 3×10^5 cells/flask, and treatment with the chemical was begun 24 h after plating. The final concentrations of DEB in the culture medium were 1.0 μg ml^{-1}, 0.1 μg ml^{-1} and 0.01 μg ml^{-1}. The half life of DEB in aqueous solution is approximately 4 d. Four days after plating, cultures were washed with PBS and refed, and fresh carcinogen was added at the same concentration. EMS at concentrations of 10^{-4} M, 10^{-5} M and

10^{-6} M was added to cultures daily because of its instability in solution. In each case, cells received a chronic exposure to chemical over a period of 6 d. Cell counts and dye exclusion studies using Trypan blue were done in triplicate 4 and 7d after initial plating. That concentration of chemical which had least effect on growth rate and viability of FA cells during the 6-d exposure period was considered to be non-toxic, and was selected for use in chromosome studies. The highest concentration of DEB found to be non-toxic to FA cells was 0.01 µg per ml medium (1.25×10^{-7} M). Figure 1 shows the growth rate of treated and untreated FA and XP cells at this concentration of DEB. For EMS the non-toxic concentration was 10^{-6} M. Higher concentrations of these compounds caused a significant reduction in growth rates of cultures, altered cellular morphology, and increased cell death.

Non-toxic concentrations of the chemicals were selected for chromosome studies primarily to study the effects of low doses of carcinogens, and to maintain the largest possible dividing pool of cells for these studies. Cells in metaphase were collected 48 h after replating and growth in carcinogen-free medium since we were interested in the residual effects of chemical exposure on the surviving and dividing population of cells. The treatment schedule used in the chromosome studies was the same as that used to determine the carcinogen toxicity.

The results of treatment with the non-toxic concentration of DEB are shown in Table 1. There was a significant increase in chromosome breakage in two strains of FA cells treated with DEB when compared with the untreated FA cells ($P < 0.001$). Qualitatively, the breakage caused by the carcinogen was similar to the chromosome aberrations which occur spontaneously in these cells. Chromatid breaks were the most common chromosome aberration in the FA cells, both before and after treatment with the carcinogen. Chemical treatment increased the incidence of chromosomal as well as chromatid aberrations. Furthermore, the breaks were not concentrated in a few cells; treated populations of FA-2 showed damage in 70% of mitotic cells treated. There was no increase in aberrations with DEB treatment of XP, trisomy 18, or normal cell cultures. The inability of DEB, at the non-toxic concentration, to induce any chromosome aberrations in these other genetically abnormal cells, was in sharp contrast to its effect on FA cells.

Treatment of FA cells with EMS also resulted in a significant increase ($P < 0.001$) in chromosome aberrations when compared to untreated FA cells (Table 2). Furthermore, no aberrations were induced in normal cells at the same non-toxic concentration of EMS. When the incidence of breakage in FA cells induced by DEB was compared with that induced by EMS, the concentration of each chemical being equivalent in its cytotoxic effects, the difunctional agent DEB was found to be a significantly more potent karyolytic agent ($P < 0.01$).

Statistical significance of the increase in breakage of treated

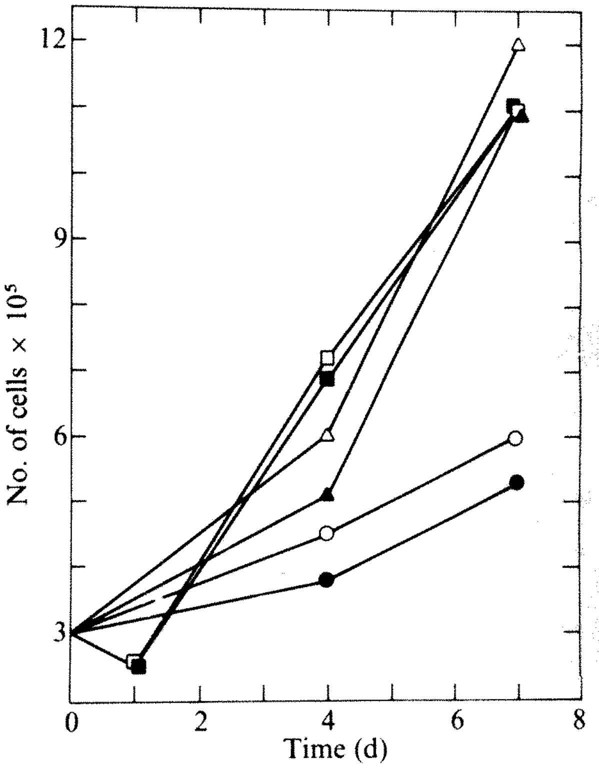

Fig. 1 Parallel cultures (open and closed symbols) showed similar increases in cell number in presence or absence of carcinogen treatment (DEB at 0.01 µg m^{-1}). Normal and trisomy 18 cells grew more rapidly, growing from 3×10^5 to $18–24 \times 10^5$ cells in 7 d. These cell strains also showed no difference in growth rate between treated and untreated cells. ○: FA-1, control; ●: FA-1 treated with DEB; □: FA-2 control; ■: FA-2 treated with DEB; △: XP, control; ▲: XP, treated with DEB.

Table 1 Chromosome breakage persisting after exposure to DEB (0.01 µg ml^{-1}) for 6 d

Cell type		Chromatid breaks	Chromosome breaks	Acentric fragments and deletions	Rearrangements*	No. of breaks per cell (50 cells per set)
NSF	Untreated	0	0	0	0	0
	Treated	0	0	0	0	0
XP	Untreated	0	0	1	1	0.06
	Treated	0	0	0	0	0
FA-1	Untreated	5	6	1	0	0.24
	Treated	16	9	7	1	0.68
FA-2	Untreated	7	2	1	0	0.20
	Treated	38	8	3	3	1.10
FA-2 (repeat)†	Untreated	16	1	1	0	0.36
	Treated	35	4	3	4	1.00
Trisomy 18	Untreated	3	1	0	0	0.08
	Treated	6	0	0	0	0.12

NSF: normal; XP: Xeroderma pigmentosum; FA: Fanconi's anaemia.
*Rearrangements included multiradials, dicentrics and translocations.
†The above strain was retested 10 months later with a different batch of chemical and at a different passage level.

Table 2 Chromosome breakage persisting after exposure to 10^{-6} M EMS for 6 d

Cell type		No. of breaks/No. of cells	No breaks	1 break	2 breaks	3 breaks	Total no. of cells with breaks
NSF	Untreated	0/100	100	0	0	0	0
	Treated	6/100	95	4	1	0	5
FA-2	Untreated	28/100	74	24	2	0	26
	Treated	61/100	53	35	10	2	47

versus untreated cells in each population was determined by the use of a t test with ∞ d.f. and the Poisson standard error. The numbers of cells with one break, two breaks, and so on, fit the expected distribution for Poisson events in both DEB and EMS treated FA cells.

These experiments separate the chromosome-damaging effects of the chemical agents from their toxic effects. We assume that selection during the chronic exposure to alkylating agents was minimal since the growth and viability of cells was not reduced. Furthermore, the same non-toxic concentrations of chemicals had no effect on either viability or rate of chromosome aberrations in the other cell strains tested. The finding of increased chromosomal aberrations in FA fibroblasts is therefore further evidence that the defect in this disease results in increased chromosome fragility in these cancer-prone individuals.

Both xeroderma pigmentosum and trisomic patients are also reported to show increased susceptibility to malignancy. Experimentally a reduction in repair synthesis has been demonstrated in XP cells after exposure to certain chemical carcinogens[13] and trisomy 18 fibroblasts have shown an increased susceptibility to SV40 transformation[14]. Although both are genetically abnormal, neither of these cell types shows the levels of chromosome aberrations found spontaneously in FA cells, which may account for the difference in chromosomal effects of the carcinogen. A biochemical basis for chromosome fragility in FA has not yet been delineated, but there is some evidence suggesting FA lymphocytes are defective in a step in DNA repair[15,16].

The persistence of chromosome aberrations in FA cells after exposure to a carcinogen suggests a mechanism for cellular evolution to malignancy. In ataxia-telangiectasia, another disease in which there is both increased chromosome breakage and increased risk of malignancy, a consistent chromosomal rearrangement of chromosome 14 was observed, which then persisted in a leukaemic clone[17]. Chromosome breaks themselves are unstable, and cells with single breaks would not contribute to the evolution of chromosomally abnormal cell clones. Exposure to chemical mutagens or carcinogens in concentrations too low to inhibit cell growth and viability but sufficient to increase the number of chromosome breaks in susceptible cells, would, however, greatly increase the probability of having more than one break per cell, and, therefore, the possible formation of a chromosome rearrangement. If such rearrangements are related to the formation of malignant cell clones, then these experiments provide evidence that the increased susceptibility to malignancy in FA patients is related to the increased susceptibility of FA cells to chromosome damage from mutagens or carcinogens.

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Natural tranquilliser in cephalopod eggs

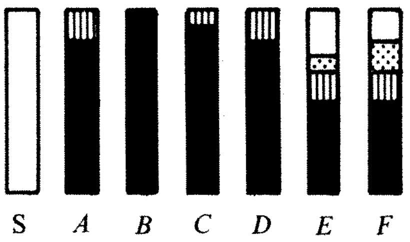
THE hatching gland of cephalopods[1-5] is fully differentiated and may fulfil its function before the embryo has reached a stage of development sufficiently advanced for life in the open sea[6,7]. It is not known why premature hatching occurs only in clearly artificial conditions. Investigations of the properties of the perivitelline fluid (PVF) of squid eggs have given us an important clue: this fluid acts as a tranquilliser on the young animals as they approach the end of embryonic development.

Our observations were made on eggs of the common squid *Loligo vulgaris*. The PVF of this species[8] is a clear, slightly viscous liquid that is hypertonic to seawater. Its volume in a single egg increases from about 0.3 ml early in development to 100–130 ml towards the time of hatching. The substances that cause water uptake through the chorion are emitted by the embryo[9]; their chemical nature is unknown. Some secretory products of the epidermal glandular cells have been demonstrated by haemagglutination tests[10]. Although the PVF which is the natural medium of the developing embryos has a composition different from seawater, embryos develop normally in sterile seawater[11]. The technique of collecting embryos and PVF is simple: the choria are opened with watchmaker's forceps and the contents are collected in glass receptacles[10].

Animals ready to hatch, which were collected in this way, became very active when placed in seawater. They swam around by jet propulsion and remained above the bottom of the receptacle, with their chromatophores active. When they were transferred to PVF, however, active swimming ceased within about 10 s. The animals sank to the bottom, where they exhibited little or no chromatophore activity. They also made little or no response to disturbance such as knocking on the receptacle, which induced an immediate escape reaction in animals kept in seawater. When returned to seawater, quiescent animals resumed active swimming within 5 s. PVF diluted 1:1 with seawater gave an effect similar to that of PVF.

Differences in pH, in O_2 content and viscosity that exist between the PVF and seawater seem likely to be responsible for the differences observed in the behaviour of the animals[12-14]. Preliminary experiments have shown that a lower pH, corresponding

Fig. 1 Pattern of activity compared with quiescence in hatchlings of *Loligo vulgaris* after 1 h of exposure to seawater (S); untreated PVF (*A*); PVF centrifuged for 1 h at 5,000 r.p.m. (*B*); PVF filtered through cotton (*C*); PVF filtered through glass fibre wool (*D*); PVF Millipore-filtered, 0.45-μ pore diameter (*E*); PVF Millipore-filtered and then heated at 100 °C for 15 min (*F*). Millipore-filtration of PVF must be preceded by the treatment indicated in *B*, *C* and *D* to avoid clogging the filter. The behaviour of the embryos in pretreated PVF was similar to that observed in *A*. Each test vessel (Petri dish, 6 cm diameter) contained 12 ml of fluid. There were 12 animals per vessel. The clear difference between S and *E* and *F* shows the presence of tranquillising agent even after filtration and heating. Open shading, active swimming; dots, hovering (compensation of sinking); vertical hatching, lying on bottom, ventral side up, with occasional jets produced; solid shading, continually quiescent.



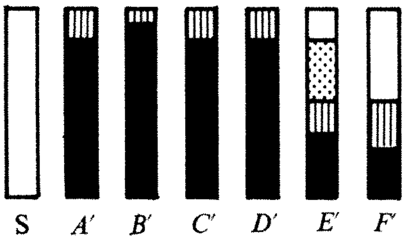


Fig. 2 Pattern of activity in hatchlings of *Loligo vulgaris* after 1 h of exposure to seawater (S) and diluted PVF at a concentration of 6.6% in seawater. *A′* is the dilution product of *A* and so on. Conditions were otherwise identical to those for Fig. 1. Shading as for Fig. 1.

to that of PVF (*p*H 7.5), does not influence swimming activity in seawater. In O_2-enriched PVF animals were activated only momentarily. Thus viscosity could be a mechanical tranquilliser.

Centrifuging and/or filtering through cotton and glass fibre wool did not alter the effect of PVF (Fig. 1 *B*, *C* and *D*). Only after the highly viscous substances secreted by the epidermal glandular cells[10] had been removed by Millipore filtration (Fig. 1*E*), did some animals become active. Yet some remained inactive. Thus there must be an actual tranquillising agent that is not eliminated by filtration; the inactive animals were presumably more sensitive to this component than the active animals. The heat stability of this remaining agent (Fig. 1*F*) suggests that it has low molecular weight. Its mild effect was complemented by the viscosity-causing substances in the untreated fluid (Fig 1*A*), which seemed to impede motion mechanically. Embryos were immobilised in seawater to which methyl cellulose (4 mg ml^{-1}) had been added. In contrast to embryos tranquillised by PVF, these embryos had expanded chromatophores. The tranquillising effect of untreated fluid was observed at concentrations as low as 1.7% (Fig. 3*A″*–*D″*), whereas Millipore-filtered fluid containing only the supposed specific tranquilliser had an effect only at higher concentrations (6.6%, Fig. 2*E′* and *F′*).

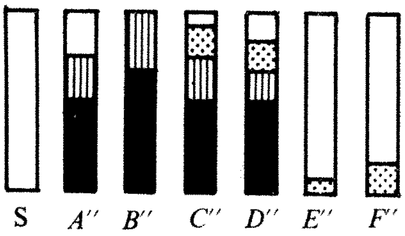


Fig. 3 Pattern of activity in hatchlings of *Loligo vulgaris* after 1 h of exposure to seawater (S) and diluted PVF at a concentration of 1.7% in seawater (compare Fig 2). Shading as for Fig. 1.

Untreated PVF of *Loligo vulgaris* also had a strong effect on small crustaceans (mysids, prawns and copepods), small fish (about 2 cm long) and young cephalopods of other species (*Sepiola* spp.). They were immobilised within a few seconds. In our experiments some died and others resumed their normal activities within 5–10 h in seawater even after exposure to PVF for 3–4 h. No effect was observed on the ciliary activity of ciliates and on the flagellar movement in spermatozoids of sea urchins (*Paracentrotus lividus*); these spermatozoids agglutinated however[10,15].

For the cephalopod embryo, the protective effect of a tranquillising mechanism is twofold: it prevents premature hatching which would mean the hatching of less viable young, and the waste of energy by muscular activity. The result is optimum conditions for the survival of well developed hatchlings and thus is part of what can be called the reproductive strategy.

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Action of papaverine and ionophore A23187 on neurulation

MICROFILAMENTS, which are thought to be activated by Ca^{2+}, are functionally related to the apical constriction of neural plate cells during neural tube morphogenesis. Papaverine interferes with Ca^{2+} flux and inhibits neurulation, disrupting microfilament structure, while ionophore A23187 which induces Ca^{2+} flux counteracts this inhibition restoring microfilaments and promoting formation of neural ridges and folds in 10 min. We conclude that microfilament-generated morphogenetic movement may be regulated, in part, by Ca^{2+} availability.

Cytokinesis[1], epithelial morphogenesis[2] and egg cortex contractility[3] all require calcium ions and the presence of microfilaments at regions of localised contractions. Cytochalasin B alters the morphology of microfilaments and inactivates their apparent capacity to produce the changes in shape cited above[4]. The relationship of calcium to these activities is not completely known; cortical contractions are induced in *Xenopus laevis* eggs and embryos by calcium injections[3], and by treatment with ionophore A23187, which promotes increased membrane permeability to divalent cations such as Ca^{2+}, while activating the cortical contractile system in the frog[5]. Papaverine, in contrast to ionophore A23187, is reported to inhibit the release of bound calcium[6]. Salivary gland morphogenesis does not proceed characteristically in papaverine, a result ascribed to interference with calcium activation of microfilaments[7]. Schroeder has proposed that microfilaments contribute to the movements of gastrulation and neurulation[5] and has demonstrated that specific arrays of microfilaments correlate with apically constricted neural plate cells[8]. We reasoned that if the timely release of calcium were causally related to the activation of microfilament contraction in neurulation, that papaverine should likewise inhibit this process and that ionophore A23187 should reactivate it.

More than 100 early neurula embryos of the amphibian *Ambystoma maculatum* late stage 13 (Harrison) were completely decapsulated and exposed to papaverine or spring water alone for a period of 10–18 h at 20 °C. The animals were distributed among spring water solutions with drug concentrations of 25, 50, 75, 150 and 300 μg ml^{-1}. Papaverine treatment disrupts neurulation; the embryos assume a spherical shape, become flaccid, and develop none of the morphogenetic features associated with the developing anteroposterior neural axis (Fig. 1*a*). Depending on the concentration, the neural groove may narrow or deepen and opposite sides come into contact but in papaverine concentrations greater than 50 μg ml^{-1}, neural ridges and folds never erupt as they do in the controls (Fig. 1*b*).

For electron microscopic observations embryos were fixed

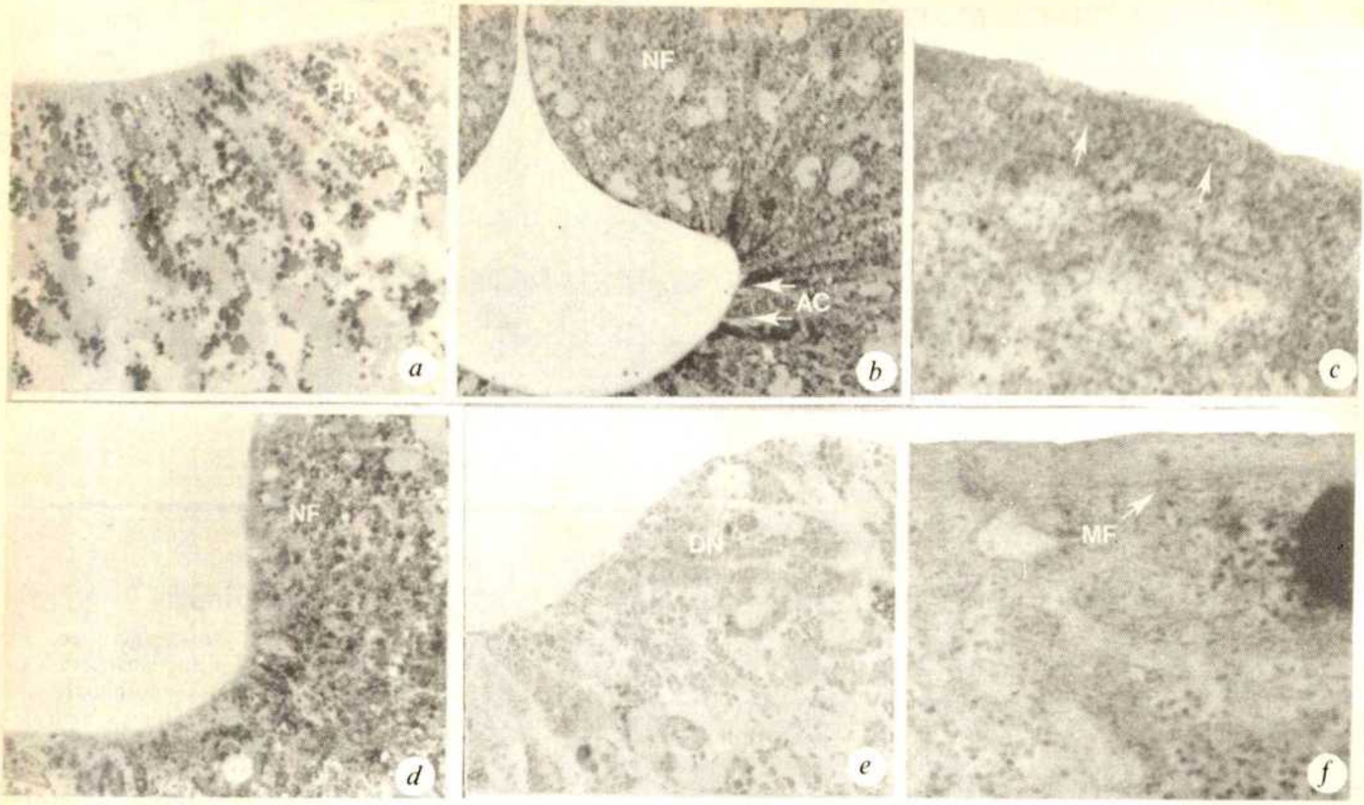

Fig. 1 *a*, Light micrograph of the neural plate of a papaverine-treated (75 μg ml^{-1}) animal. The neural ridges and folds fail to form, 163. PR, presumptive ridges. *b*, Light micrograph of a neural fold of comparable-aged control animal in spring water. Arrows (AC) indicate the position of apically constricting microfilaments of neural fold cells, 325. NF, neural fold. *c*, Electron micrograph of a neural ridge cell of a papaverine-treated (75 μg ml^{-1}) animal. Note the apical band of linear microfilaments is not present (arrows), 23,400. *d*, Light micrograph of a neural fold which developed on returning a papaverine-treated (75 μg ml^{-1}) animal to spring water for 12 h, 241. NF, neural fold. *e*, Light micrograph of a developing neural fold of a papaverine-treated (75 μg ml^{-1}) animal removed to ionophore A23187 (25 μg ml^{-1}) for 5 min, 241. DN, developing neural fold. *f*, Electron micrograph of a neural ridge cell of a papaverine-treated (75 μg ml^{-1}) animal removed to ionophore A23187 (25 μg ml^{-1}) for 5 min. Note the restoration of the apical band of microfilaments, 48,750. MF, microfilaments.

with 2.5% glutaraldehyde in Millonig's buffer and postfixed in 1.0% buffered osmium tetroxide. Specimens were embedded in Epon, mid-trunk sections were cut with a Porter–Blum MT2-B ultramicrotome and examined with a Philips EM300 operating at 60 kV. Exposure to papaverine results in distinctive ultrastructural alterations of neural ridge cells. The dense band of microfilaments beneath the apical plasma membrane in control cells is not present in either grazing or vertical sections (Fig. 1*c*). Pigment granules are arrayed with unusual linearity beneath the plasmalemma. The normally planar apical surface develops blunt projections, mitochondria display vacuoles and loss of cristae, and gaps appear between the usually tightly apposed cells.

If papaverine treatment does not exceed 24 h, washing the animals and returning them to spring water results in a reinitiation of neurulation, with neural ridges and folds forming (Fig. 1*d*) and fusing within 1 d. This reversible inhibition by papaverine and its putative interference with the requisite calcium flux of neurulation provided us with a model system with which to assess the import of Ca^{2+} in these events by counteracting papaverine's stabilisation of calcium with the cation ionophore A23187. Stock solutions were prepared with a 1 : 3 solvent mixture of *N,N*-dimethyl formamide and 95% ethanol. Working solutions of 10 and 25 μg ml^{-1} were made by dilution with spring water. Control series were run in spring water and diluted solvent solutions and showed no disruption of morphogenesis.

After washing, a 10-min exposure of papaverine-inhibited animals (75 μg ml^{-1} to 25 μg ml^{-1} of ionophore A23187) results in eruption of the neural ridges (Fig. 1*e*), which then rapidly form discrete folds, not always in their normal medial orientation. More than 10 min of ionophore treatment promotes cell disaggregation and the subsequent collapse of the entire embryo. Electron microscopic observations reveal that dramatic fine structural changes occur during the short time in the A23187 solution. The subplasmalemmal dense band of microfilaments is reconstituted (Fig. 1*f*), with the pigment granules redistributed throughout the cytoplasm. Surface projections and gaps between the cells are not seen, specialised cell junctions (that is, desmosomes) are evident, and the mitochondria appear intact with numerous cristae. Microtubules, which were also seen in papaverine-treated cells, are arrayed along the vertical cell borders and within the apical dense band.

We have recently proposed a model of neurulation based on cumulative evidence of the involvement of mechanical forces and a directional guidance system together with our reports of supracellular participation of cell surface material (CSM) in the alignment and fusion of the neural folds[9]. Cellular movements and shape changes during neurulation apparently result from selective constriction and elongation. The constrictive force has been attributed to microfilaments[8], while elongation seems related to the presence of microtubules[10–12]. If the activity of microfilaments during the eruption and convergence of the neural ridges and folds depends on the continued availability of endogenous calcium, deactivation by papaverine should be counteracted by the effect of calcium ionophore A23187. Our experiments confirmed this, demonstrating an ionophoric induction of the morphogenetic movements of neurulation. The precise accomplishment of complex morphogenetic events such as this depends on appropriate temporal cues which, in turn, must interrelate with the spatial location and structural differentiation of diverse cells and tissues. Our data implicate calcium as an instructive agent in these activities. The experiments of Barth and Barth[13,14] earlier suggested the involvement of ions in amphibian development, most notably Ca^{2+} during neurulation, when its incorporation was shown to be markedly raised.

During embryogenesis, microfilaments develop concomitantly with cellular motility and alterations in shape[11,12,15]. It has been suggested that these organelles accommodate cellular constriction and movement in a manner similar to the actin and myosin filaments in the sliding hypothesis proposed for muscle cells[16]. By analogy with muscle cells, non-muscle cells might also require Ca^{2+} for contractile activation[15]. The results presented here provide support for this idea and agree with the findings of Schroeder and Strickland[5] regarding ionophore-mediated Ca^{2+} release and its subsequent triggering of the cortical contractile system. Our electron microscopic observations indicate that papaverine disruption of normal Ca^{2+} ion mobility affects cell junctions, microfilaments and mitochondria, all of which are involved in cell movement. Microtubules, which are thought to be responsible for the directionality and structural stability of moving cells or tissues, do not seem to be affected by papaverine treatment but more subtle effects cannot be precluded by our experiments. It should be noted that *in vitro* experiments have demonstrated the relation of Ca^{2+} and microtubule metabolism; high concentrations of Ca^{2+} inhibit their polymerisation[17]. Microtubules do not, however, seem altered by ionophore treatment. It may be, therefore, that they are normally constituted and functioning before microfilament activation, so that the high titre of Ca^{2+} induced by the ionophore only stabilises them, and does not affect their assembly. Further work is needed on this point.

Correlations between cell movement and CSM accumulation during gastrulation and neurulation have been established[9,18,19]. As the neural axis forms extracellular materials provide an adhesive link between the convergent folds. This CSM is negatively-charged, stains avidly with Alcian blue and may, as in related amphibian systems[20], be polymerised and distributed at the cell surface by interactions involving Ca^{2+}. In summary, the present study implicates Ca^{2+} as a mediator of microfilament contraction and suggests the possibility that selective release of this ion may be involved in the mechanism by which non-muscle cells regulate their movement

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Loss of a critical neutral protease in ageing WI-38 cells

THE limited lifespan of normal human diploid cells *in vitro* seems to be related to the number of mitotic divisions the cells have undergone rather than to passage of time[1-3]. In spite of considerable research[3-7] it is still unclear what biochemical events actually mediate the cell death or more accurately the absence of growth. In other systems it has been postulated that regulation of growth may be mediated by activity at the cell membrane. Specifically it has been suggested that cellular division is controlled in part by membrane mediated cell contact, and proteases and lysosomal enzymes have ben implicated in the control of mitosis and growth[8-11]. Thus cell surface events may be either primary or secondary events mediating cell ageing. Acid hydrolases have been postulated as modifying the cell surface in sublethal autolysis, electrophoretic mobility of cells gives an indication of cell surface chemistry and neutral proteases have been implicated in modification of cell external surfaces[9-15]. We now demonstrate that two cell surface parameters are greatly affected by *in vitro*

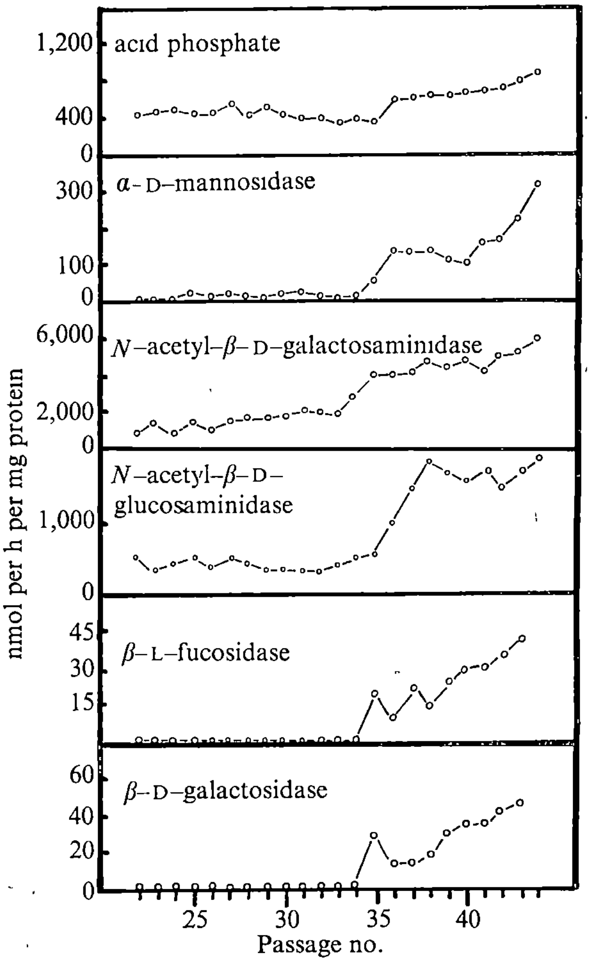


Fig. 1 Activity of six acid hydrolases as a function of passage of time in WI-38 cells. Values are means from 4 separate determinations. Glycosidase and acid phosphatase activity was determined as given previously[12,16], on 0 1% Triton X-100 homogenates of the cells using *p*-nitrophenyl substrates. Details of cell culture and definition of passage time are given in the text. In this and all succeeding figures the passage points are not connected because it is not clear that the activities of successive passages are truly a continuous function. It should be noted that the inability to detect activities of several lysosomal enzymes in young and middle aged cells merely reflects the relative insensitivity of the method of assay and does not preclude low levels of these enzymes being present.

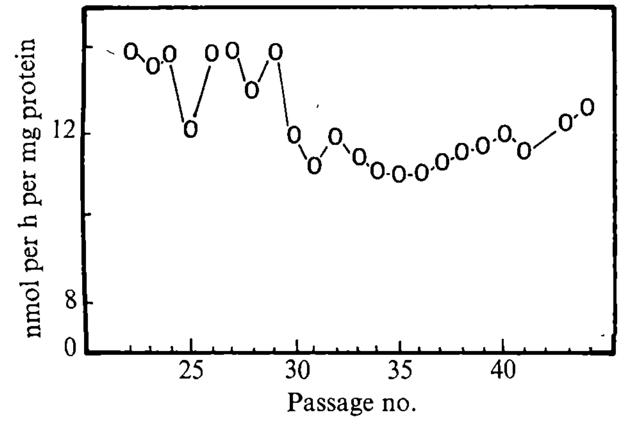


Fig. 2 Activity of cathepsin-like proteolytic activity at *p*H 3.4 as function of passage time in WI-38 cells. Values are means from 4 separate determinations. Proteolytic activity at *p*H 3 4 was determined as given previously[17,18] on 0 1% Triton X-100 homogenate of the cells using ^{3}H-acetyl haemoglobin as substrate. ^{3}H-acetyl haemoglobin was prepared as described previously[17,18]. ^{3}H-acetic anhydride (400 Ci mol^{-1}) was reacted with the type I beef blood haemoglobin. The material was purified to a specific activity of 162 c.p.m. pmol^{-1} based on a molecular weight of 68,000 using the counting procedures described here. The specific activity of the material was 300 Ci mol^{-1}. Routinely in the assay systems described below, 50 μl (136 μg ^{3}H-acetylated haemoglobin, 2 nmol, 324,000 c.p m.; 0.6 μCi) of the ^{3}H-acetylated haemoglobin was added per assay. Routinely 100 μl of the 0 1% Triton X-100 homogenates (between 50 and 100 μg protein) were added to 100 μl 1.35 M acetic acid, 0.2 M ammonium sulphate buffer (*p*H 3.4) As the substrate, 50 μl of ^{3}H-acetyl haemoglobin (2 nmol) were added. This mixture was incubated for 1 h at 37 °C in a Dubnoff metabolic shaker. The reaction was terminated by placing the assay tubes in an ice-water bath (0–4 °C) and adding 100 μl of 2.5% haemoglobin and 50 μl of 50% trichloroacetic acid (TCA) The precipitated material was removed by centrifugation at 5,000*g* for 5 min, and an aliquot of the supernatant fluid was plated on a glass fibre filter; the radioactivity was determined by counting in a liquid scintillation counter[17,18]. Activity is expressed as nmol haemoglobin degraded per h per mg protein.

ageing: activity of a *p*H 7.8 protease and cell surface net charge as measured by electrophoretic mobility. It should be emphasised that the small standard deviations for the electrophoretic mobility data reported herein are a function of the large *n* (number of independent observations) and do not preclude population heterogeneity.

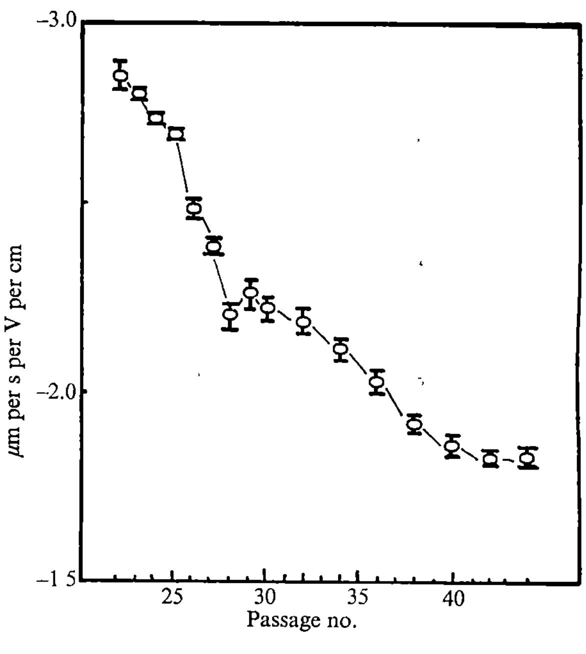
WI-38 cells at various passages were kindly provided by Dr L. Hayflick. To avoid confusion the culturing conditions used for determining passage number will be precisely described. Cells were grown, maintained, subcultivated, and passage number determined exactly as given on p. 615 of ref. 2. The number of cells was divided in half every 3 d and then replated in fresh Eagle's basal medium (Gibco; G-13 powdered media) with 10% of calf serum, penicillin (500 U ml^{-1}) and streptomycin (1 g^{-1}). Trypsin at a concentration of 0.025% was used to remove the cells from the monolayer. Thus passage number is determined herein by the "2 : 1 cell split method"; furthermore, all cells used for the experiments herein were grown on glass. Finally, it is important to describe the behaviour of the cells in our hands. To all outward appearances the cells grew normally until passage 35/36, when a definite decrease in the density of the cell population occurred at each harvest and subcultivation. By passage 44 the cells were so sparse it became impossible to obtain enough cells for all the biochemical procedures. Thus the cells ceased to grow at passage 44 (these cells would thus be "end phase III" cells using the nomenclature of others[6]). We did manage to carry a limited number of cells to passage 48 for one experiment but that required a great number of flasks and large quantities of medium. For all analyses (not passages) cells were washed by centrifugation at 500*g* for 3 min and resuspended in the above described medium without the serum before use. This procedure took 20 min.

Acid phosphatase (EC 3.2.3.2), α-D-mannosidase (EC 3.2.1.2.4), *N*-acetyl-β-D-glucosaminidase (EC 3.2.1.30), *N*-acetyl-β-D-galactosaminidase (EC 3.2.1.–), β-L-fucosidase (EC 3.2.1.38) and β-D-galactosidase (EC 3.2.1.23) were assayed on 0.1% Triton X-100 homogenates of the WI-38 cells as described previously using *p*-nitrophenyl substrates[12,16]. Proteolytic activities at *p*H 3.4 and 7.8 (refs 17 and 18) were assayed on 0.1% Triton %-100 homogenates of the WI-38 cells using ^{3}H-acetyl-haemoglobin as substrate. Electrophoretic mobility of the cells was measured as described previously[18–20]. Protein was determined by the method of Lowry *et al.*[21].

The data of Fig. 1 show that each of the acid hydrolases (thought to be primarily but not exclusively lysosomal) increased as the cell passage number increased. A rather marked increase occurred at passages 34–37 and the increase continued until passage 44; activity of each of the six enzymes studied per mg protein reached a maximum at passage 44. Passage 36 was the passage in which the cells began to grow less densely and this coincides with the increases in acid hydrolase activity. A sharp increase in activity occurred for β-D-mannosidase, which from passage 22 to passage 34 hovered around 0 (undetectable) activity and in β-D-galactosidase and β-L-fucosidase both of which had total 0 (undetectable levels) before passage 35. With regard to *in vitro* ageing it may be as important to determine why those enzyme levels are so low (0) before passage 35 as it is to determine why the increase occurs at late passages until passage 44. Activity levels of acid hydrolases ("lysosomal hydrolases") have been reported in general to increase on ageing *in vitro* (see for example refs 6, 7, 22, 23) and we now confirm many earlier observations.

Fig. 2 gives results of the *p*H 3.4 activity protease

Fig. 3 Electrophoretic mobility of WI-38 cells against passage number of the cells. Values are means ± 1 s.d. for approximately 120 independent particles at each point. Electrophoretic mobilities were determined as previously described[18,20]. Values are for cells measured in 0.0145 M NaCl, 4.5% sorbitol, 0.6 mM $NaHCO_3$ (*p*H 7.2±0.1) at 25 °C±0.1, as previously described[18,20]. Ionic strength = 0 015 g ions l^{-1}. These data reflect population net negative surface charge. Because of the very high number of independent observations (> 120) the standard deviations are small.



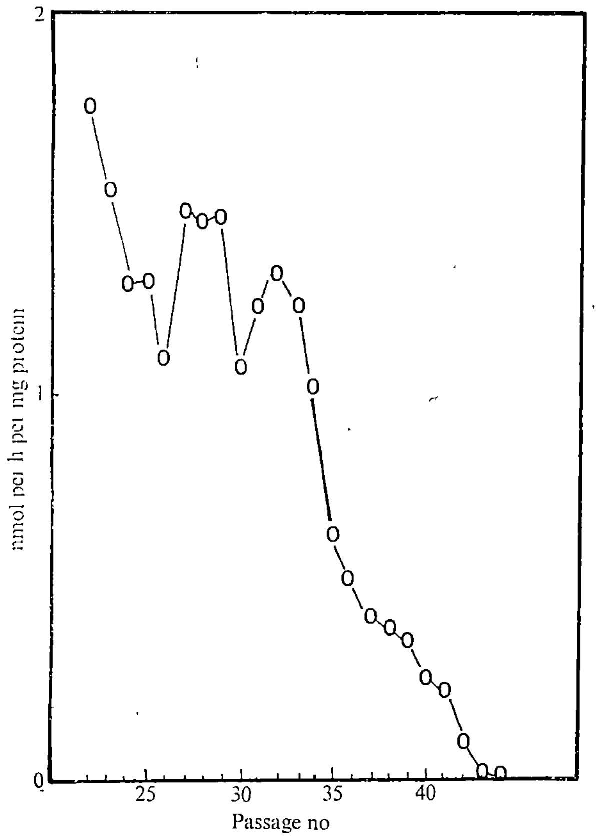


Fig. 4 Activity of trypsin-like proteolytic activity at *p*H 7 8 as a function of passage time in WI-38 cells Values are means from 4 separate determinations. Proteolytic activity at *p*H 7.8 was measured exactly as given in Fig. 2 except that 0 1 M phosphate buffer (*p*H 7.8) was used in the assay in place of the *p*H 3.4 buffer[15,18].

(cathepsin-like) thought by many to be of lysosomal origin. Although high activity levels of this enzyme are present in the WI-38 cells the enzyme activity seems to only decrease slightly at passages 30–40 and increase slightly at passages 40–44.

The electrophoretic mobility data shown in Fig. 3 are unique. The data presented show that the net negative surface charge decreases from -2.82 ± 0.04 at passage 22 to -1.82 ± 0.02 at passage 44. The decrease is most pronounced at passages 25–28 and levels off at passages 42–44. On the basis of work on other mammalian cells and organelles[18–20], it is tempting to speculate that the decrease is related to a loss of external surface *N*-acetyl-neuraminic acid; such conclusions must, however, await chemical data which require large numbers of cells.

The most interesting observation is shown in Fig. 4. The *p*H 7.8 neutral protease activity essentially disappears at passages 43 and 44. Specifically, the activity of the enzyme goes to 0 or non-detectable activity at passage 43/44 from a passage 22 activity of 1.68 nmol per h per mg protein. To test this finding further in separate experiments passage 27 cells were grown from frozen stock (liquid N_2) and compared with cells that had grown out to passage 48. The passage 27 cells had 1 5 nmol per h per mg protein of *p*H 7.8 protease activity and the passage 48 cells had 0 nmol per h per mg protein of *p*H 7.8 protease activity (passage 27 cells had 427 nmol per h per mg protein of acid phosphatase activity and passage 48 cells 917 nmol per h per mg protein of acid phosphatase activity; see Fig 1). This neutral protease is thus present in the rapidly dividing early passage WI-38 cells and absent from the senescent late passage WI-38 cells.

To ensure that the loss of *p*H 7.8 activity was not due to differential binding between phase II and phase III of the enzyme used to remove the cells from the monolayer, the following experiments were performed. Using 0.0025, 0.025, 0.25 or 1% trypsin or removal of the cells with a rubber policeman rather than the usual 0.025% trypsin for passage 27–30 and passage 42–44 cells did not at all affect the results. That is, the passage 27–30 cells had the relatively high activity exactly as shown in Fig. 4 whereas the passage 42–44 had no measurable activity as given in Fig. 4. Thus the data shown in Fig. 4 are not an artefact of the trypsin used in the passage procedure

In conclusion, three parameters which are either directly or indirectly implicated in plasma membrane events are altered as cells age *in vitro* Bradley *et al.*[24] have recently determined that only at the last population doubling of WI-38 cells do these cells have increased susceptibility to proteolysis of total proteins. The present results indicate that such proteolysis probably proceeds through the *p*H 3.4 or other protease activity but not the *p*H 7.8 protease since more activity remains at the last population doubling. Furthermore the fact the total protein proteolysis does not occur until this last population doubling of WI-38 may indicate this is merely a terminal reaction while surface alterations such as decreased electrophoretic mobility and loss of activity of a potentially critical surface active *p*H 7.8 protease may indeed initiate these terminal events.

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Voltage sensitivity of acetylcholine currents in *Aplysia* neurones in the presence of curare

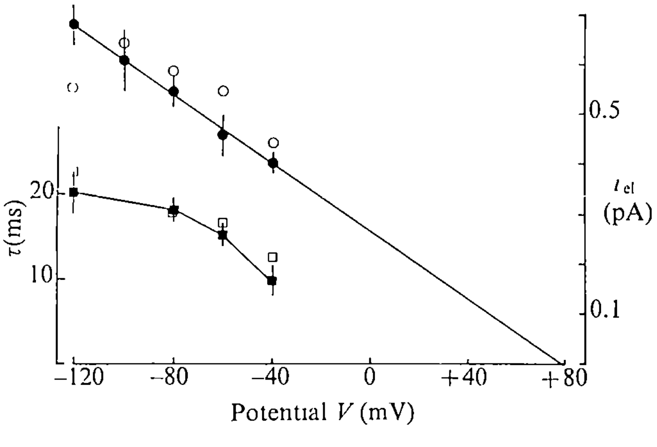
AT the frog neuromuscular junction, acetylcholine (ACh) induces fluctuations of the membrane potential[1] or current[2], from which one can evaluate the intensity and the duration of the 'elementary current' flowing through individual ionic channels. The observation that the duration of the elementary event increases with hyperpolarisation[2] has provided an explanation for the fact that, when ACh is applied at the end plate in long pulses, the total ACh current increases more with hyperpolarisation than does the elementary current[3,4]. By studying the currents induced by ACh on *Aplysia* neurones, we have observed a similar phenomenon. But we have discovered that in the presence of curare the total ACh current increases little or even decreases with hyperpolarisation, while the elementary current behaves as in control conditions. The analysis of this phenomenon suggests that, in our preparation, most of the binding of curare does not occur on the free ACh receptor, as in the frog, but rather follows the binding of ACh. It also suggests that the binding of curare is voltage dependent.

We selected for our study a group of small cells (about 60 µm in diameter) situated at the posterior pole of the right pleural ganglion. These cells respond to ACh by a purely excitatory response (completely abolished by hexamethonium 10^{-3} M, see ref. 5). They have a high input resistance (10–40 MΩ) and display very little spontaneous synaptic activity. They were voltage-clamped using two low-resistance (2–10 MΩ) microelectrodes. Temperature was set at 12 °C. ACh was applied iontophoretically from pipettes filled with 1 M AChCl in pulses of about 30 s. Spectrum analysis of the current fluctuations was done on a Hewlett Packard Fourier analyser. Background noise in the absence of ACh was always small enough to make the subtraction of spectra unnecessary

We first compared the voltage sensitivity of the 'elementary ACh current' to that of the 'total current'. The elementary current, i_{el}, was calculated as the ratio of the variance over the mean of the total ACh current[1,2]. It increased roughly linearly from −40 to −120 mV (Fig. 1). The total current also increased linearly between these values (Fig. 2), but the increase was larger. For example, between −40 and −80 mV, the elementary current was multiplied by 1.35, whereas the total current was multiplied by 2.5. A clue to this difference was given by the analysis of the ACh noise power spectrum. This spectrum could be fitted with a single Lorentzian component. The average life time of the channels, τ, was calculated from the cut-off frequency, f, as $\tau = 1/2\pi f$ (refs 1 and 2) τ increased with hyperpolarisation· it was about 10 ms at −40 mV and 18.5 ms at −80 mV (Fig. 1). This increase accounts for the difference in the voltage dependences of the elementary ACh current and of the total ACh current.

We then repeated the experiments in the presence of tubocurarine (TC) (5×10^{-5} M). The values of i_{el} and τ were unchanged (Fig. 1). On the other hand, curare drastically modified both the time dependence and the voltage dependence of the total ACh current. Fig. 3 shows the responses to ACh at two potentials, before and after application of curare (5×10^{-5} M). In normal seawater (Fig. 3*a*) the response was larger at −80 mV than at −40 mV. In the presence of curare (Fig. 3*b*), however, the early part of the response increased with hyperpolarisation but the later part was nearly unchanged. The responses of Fig. 3*b* (in the presence of curare) were obtained with higher doses of ACh than those of Fig. 3*a*. When the ACh current was not increased after curare treatment comparison of the responses with the controls showed that the initial part of the ACh response was changed little or not at all by curare, which acted only on the later part of the rising phase. This suggests that most or all of the curare blocking action depends upon prior activation of the receptor by ACh.

Fig. 1 Elementary ACh current as a function of membrane potential in control experiments (●) and in the presence of 5 × 10^{-5} M curare (○), and average lifetime of the opened channels calculated from noise power spectra in the absence (■) and in the presence (□) of curare The control points are the mean of 5–19 experiments with error bars corresponding to twice the s e.m. Points in the presence of curare are the mean of 1–3 experiments



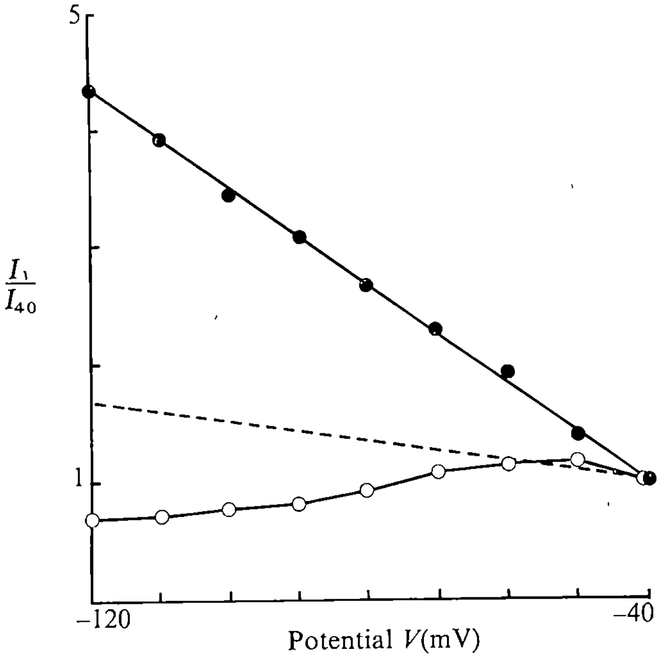


Fig. 2 ACh plateau current as a function of voltage normalised to its value at −40 mV. Control (●). the current increases with hyperpolarisation. In 10^{-4} M TC (○), the current decreases with hyperpolarisation beyond −50 mV ----, Variation of i_{el} replotted from Fig. 1.

Experiments like that of Fig. 3 are made difficult by the long recovery periods needed to compare adequately successive ACh responses. Another way of analysing the voltage dependence of the ACh currents is to modify the potential during a given ACh application. Figure 4 shows such an experiment. In control conditions, when the membrane potential was brought from −40 to −80 mV, the ACh current showed a two-step increase: an instantaneous one (expected from the increased driving force), then a more progressive one. Repolarisation brought similarly a two-step decrease. This behaviour, similar to what has been observed elsewhere[6–8], is what is expected from the variations of τ with V. More unexpected, however, is the behaviour observed under curare. There (Fig. 4*b*) when the membrane potential was brought from −40 to −80 mV, the increase in current was followed by a decrease which eventually brought the steady ACh current close to that measured at −40 mV. Fig. 2 shows the voltage dependence of the steady current obtained from such experiments.

The 'curare-induced relaxation' was not of a pure exponential form. It was markedly speeded up as the concentration of curare increased from 10^{-5} M to 2×10^{-4} M. Its kinetics seemed specific for curare, since preliminary experiments showed that voltage-dependent phenomena qualitatively similar to those of Figs 2 and 4 can also be obtained with a variety of cationic blockers of the ACh response (hexamethonium, procaine, atropine, tetraethylammonium) but that, for a given blocking effect, the kinetics of the relaxation varies markedly from one antagonist to the other.

The comparison of our results with those obtained at the frog neuromuscular junction indicates a number of differences. If one assumes that the linearity of the *I–V* curves for the elementary current (Fig. 1) is maintained at depolarised membrane potentials, the extrapolation of this curve leads to a reversal potential of +80 mV, and to an elementary conductance of $3.3 \times 10^{-12}\ \Omega^{-1}$. The value of +80 mV is close to the value of the Na^+ equilibrium potential in *Aplysia* neurones[9]. It is therefore tempting to suggest that the ionic channels opened by ACh in *Aplysia* are pure Na^+ channels, in contrast to the Na^+–K^+ channels of the frog's endplate. This difference in selectivity could be linked with the smaller value of the elementary conductance in *Aplysia*.

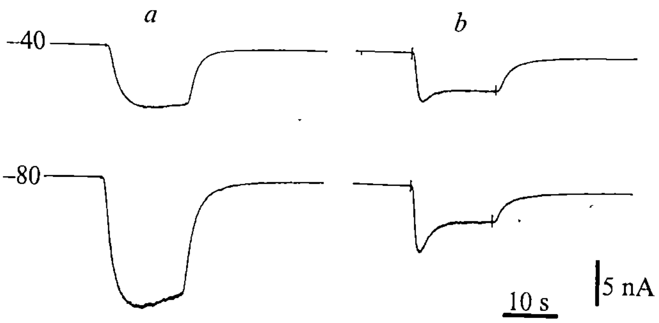


Fig. 3 ACh currents obtained at −40 and −80 mV in control conditions (*a*) and in 5 × 10^{-5} M TC (*b*). The position of the ACh electrode was not changed during the experiment, but the intensity of the iontophoretic current was increased in *b*. Note the noise increase associated with the ACh current. Calibrations, 5 nA and 10 s.

The receptor linked with the excitatory effects of ACh in *Aplysia* is known to be different from the endplate's receptor[5] and it is therefore not too surprising that curare does not act as a simple competitive inhibitor of ACh in *Aplysia*. In fact, the effects of curare in *Aplysia* resemble in many respects those of local anaesthetics on the endplate: both the effects on the shape of the ACh response and the reduction of ACh conductance with hyperpolarisation have been observed under procaine or xylocaine in the frog (refs 10–12 and see ref. 13). One interpretation of these effects of local anaesthetics supposes binding of the drug either to an 'activated' ACh receptor complex[11,12] or to the open channel itself[14]. This type of interpretation can be applied to the effects of curare in *Aplysia*. For example, if the reaction of ACh (A) with its receptor (R) is written

$$A + R \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} AR$$

where AR corresponds to the active (open) configuration of the ACh receptor complex, then the main action of curare could be written

$$AR + C \underset{\delta}{\overset{\gamma}{\rightleftharpoons}} ARC$$

ARC being assumed to be non-conducting.

One has to account, however, for an important point: procaine modifies the shape of the ACh noise power spectrum in the frog[15] whereas curare has no such effect in *Aplysia* (Fig. 1). The model predicts a shortening of τ in the presence of the antagonist, C. But the amount of shortening will depend on the relative values of $\gamma.[C]$ and α, assuming that both $\beta.[A]$ and δ are small compared to α. Thus, if the rate constants are such that in our experiments

$$\gamma.[C] \ll \alpha$$

curare will not modify τ appreciably.

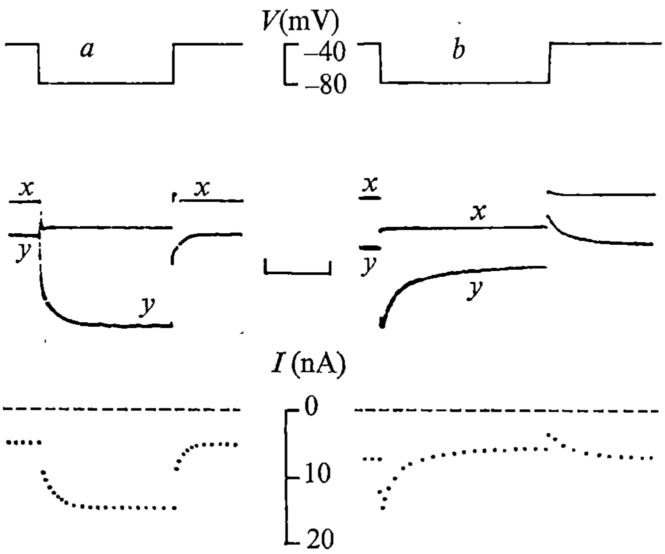


Fig. 4 ACh current response to a voltage step in a control experiment (*a*) and in 10^{-4} M TC (*b*) (from two different cells). Top traces: the voltage was stepped from a holding value of −40 mV to a test value of −80 mV and back. Middle traces: currents obtained before (*x*) and during (*y*) the application of ACh. Lower traces: ACh current (*I*ACh) obtained by subtraction of *y* from *x*. As in Fig. 3, inward currents are downwards. Time calibration: 100 ms for *a*; 2 s for *b*. A relaxation in the same direction as in *a* can barely be seen at the beginning of the current at −80 mV in *b*; with a faster sweep speed, it was observed to be very similar to the relaxation seen in *a*. The relaxations in *a* could be fitted with simple exponentials. The time constants of these exponentials (11 ms at −40 mV; 17 ms at −80 mV) compare well with the values of τ obtained from noise analysis (see Fig. 1).

We can also account for the fact that the blocking effect of curare increases with hyperpolarisation by assuming, in the above model, that the binding of curare on AR is favoured by hyperpolarisation. Such voltage sensitive binding[12] could occur if the binding site for curare were somewhat inside the hydrophobic part of the membrane, in a region sensitive to the externally applied potential.

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Strontium–calcium substitution in synaptic transmission in turtle retina

RECENT electrophysiological and morphological studies strongly suggest that the synaptic transmission between photoreceptors and horizontal cells in the vertebrate retina is chemical in nature, although the anatomical appearance of the neuronal contacts does not strictly conform to that of conventional chemical synapses[1–3] It has been shown that changes in the ionic composition of the extracellular medium, such as lowering calcium, increasing magnesium or adding cobalt, that in the chemical synapses are known to block the release of transmitter[4,5], abolish the response to light of horizontal cells without reducing that of photoreceptors[6–8]. The present report provides additional evidence that the synapses between photoreceptors and horizontal cells have properties characteristics of conventional chemical synapses. It is known that in the chemically transmitting synapse, strontium ions can substitute calcium ions in the process of transmitter release[9–11]. I have studied the effects of calcium–strontium substitution on the intracellularly recorded responses to light of horizontal cells and cones in the retina of the turtle (*Pseudemys scripta elegans*).

After draining the vitreous humour, the isolated eyecup, with the retina still attached to the pigmented epithelium, was mounted in a perfusion chamber[12] where a modified Ringer solution continuously flowed over the vitreous side[13]. Intracellular recordings were made with glass micropipettes filled with 4 M potassium acetate. The light stimuli were the reduced images of circular apertures, the diameter of which on the retina could be varied from 1,200 to 100 μm. The light source was a quartz iodine lamp (Riluma 100 W) and neutral density filters were used to reduce light intensity. The total flux density of unattenuated light at retinal level was about 1.2×10^4 erg cm^{-2} s^{-1} with wavelengths from 450 to 900 nm. Receptor and horizontal cell responses were identified by the criteria given by Baylor and Fuortes[14].

The effects of calcium–strontium substitution on the responses to light of a horizontal cell are shown in Fig. 1. Adding Sr^{2+} to the retinal medium restores the light response of the cell abolished by calcium removal (Fig. 1*A*). The shape of transients at the light onset and offset, however, differs in the presence of Sr^{2+} with respect to the control Ringer (Fig. 1*B*). Similar changes are observed in the depolarising transient at the light offset, both in strontium and EDTA Ringer, and so they seem to depend on the absence of calcium ions, while the prolongation of the depolarising phase at the light onset is seen only in the absence of strontium. Further differences between the response to light of horizontal cells in control Ringer and in calcium-free strontium solution, are illustrated in Fig. 2, in which the stimulus response functions (*V*/log *I*) are plotted for the same horizontal cell in the two conditions. The amplitude of the response to light in the presence of Sr^{2+} is lower than that in control Ringer over most of the intensity range (see in particular inset *a*) but approaches the control amplitude at the highest intensity (see inset *b*).

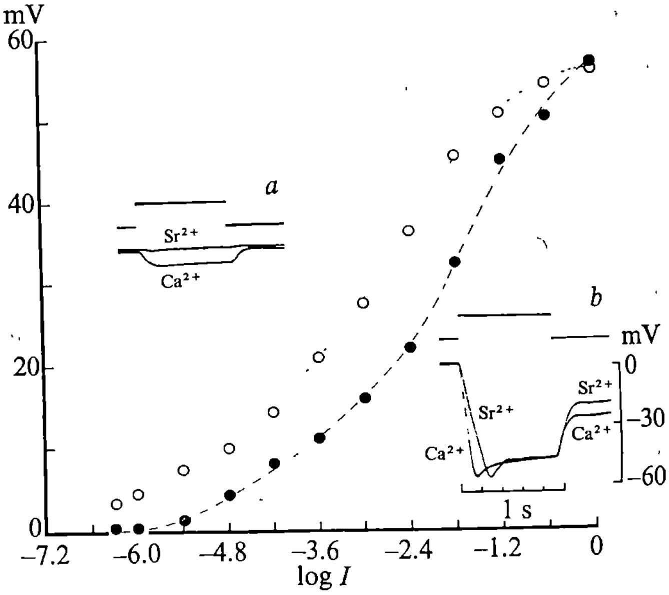

Fig. 2 Peak amplitude of the response of a horizontal cell in control Ringer (○) and during perfusion with calcium-free strontium solution (●), plotted as a function of light intensity. Records in insets *a* and *b* are the responses obtained in the two conditions to light attenuated by 5.4 log units (*a*) and to the maximum available energy (*b*). Area illuminated was 1,200 µm in diameter.

These differences in the response of the horizontal cell can be interpreted as being due, at least in part, to the action of the ionic changes on the responses of presynaptic neurones—that is, cones. Indeed, control experiments have shown that during the perfusion with low calcium–strontium solution the *V*/log *I* curves of the cones and their depolarising transients at the end of the stimulus are modified in a way comparable to that observed in the horizontal cells. Similar modifications are also observed during perfusion with low calcium–EDTA Ringer, again suggesting that they

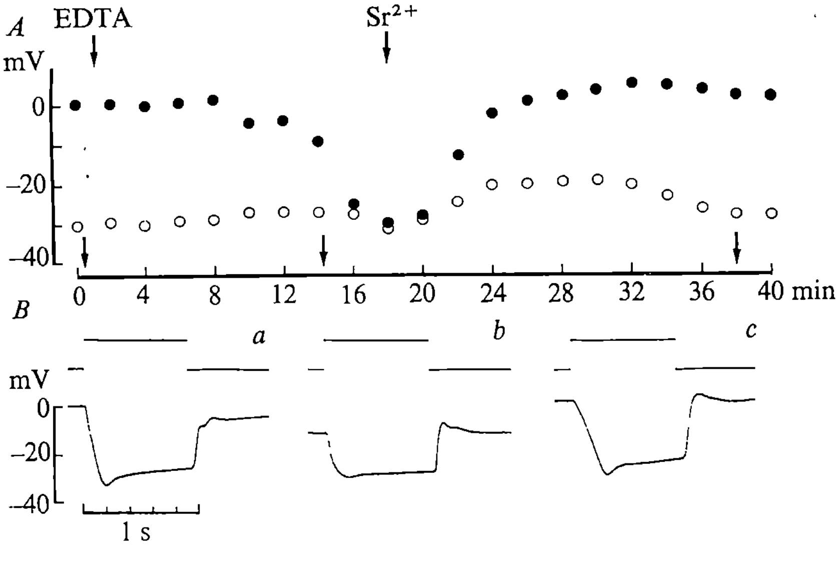

Fig. 1 *A* and *B*, Effects of calcium–strontium substitution on response to light of a horizontal cell. *A*, ●, Level of membrane potential in darkness; ○, peaks of responses to light. *B*, Zero level of membrane potential is arbitrary and indicates the level of membrane potential in darkness during the perfusion with control Ringer. During the experiment the retina was stimulated with long pulses of light (see the trace above each record in *B*) at a constant rate (0 1 s^{-1}). Light intensity was attenuated by 2.4 log units with respect to the maximum available energy; the area illuminated was 1,200 µm in diameter. Arrows on abscissa in *A* indicate the time at which the records in *B* were taken. EDTA indicates the application of a Ringer solution without calcium containing 2 mM l^{-1} of EDTA, and Sr^{2+} the application of a Ringer solution without calcium, containing 2 mM l^{-1} of strontium ions.

depend on the absence of calcium ions from the medium rather than on the presence of strontium (see also ref. 8). On the contrary, the time course of the hyperpolarising transient of the cones at the onset of the stimulus is not significantly different in the three conditions.

Previous work has shown that the transmission of electrical signals from photoreceptors to horizontal cells depends critically on the presence of calcium in the extracellular fluid[7] and is blocked either by excess magnesium[6-8] or by the presence of cobalt ions[7]. In this paper I have demonstrated that the addition of strontium ions to the retinal medium restores the response of horizontal cells to light abolished by calcium removal. All these properties are characteristic of the chemical transmission in conventional synapses and therefore these results strongly support the chemical nature of the signal transmission from photoreceptors to horizontal cells.

As to the modifications in the time course of the depolarising transient and in the $V/\log I$ curves observed in the response of the horizontal cells during the perfusion with calcium-free strontium Ringer, these seem to depend on similar changes in the behaviour of the cones in the same conditions. On the contrary, the prolongation of the time course of the hyperpolarising onset in the horizontal cells responses has no counterpart in cone responses At the neuromuscular junction Sr^{2+} ions are known to increase, by way of a postsynaptic effect, the duration of transmitter action. If one assumes that the hyperpolarising responses to light of horizontal cells result from a reduction in the release of a depolarising transmitter[15] continuously flowing in darkness from the receptor pedicle, increasing the duration of transmitter action would result in a slower time course of the hyperpolarising phase at the light onset.

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Postexposure serum prophylaxis of neonatal herpes simplex virus infection of mice

PERINATAL infections with herpes simplex virus (HSV) cause death or permanent disability in most reported cases[1-5]. In theory, control of this disease by immunotherapy might be feasible, much like postexposure serum prophylaxis of rabies, since the time of HSV infection in the birth canal is usually known and antibody can be administered soon thereafter. Treatment with antibody has not been encouraged, however, because (1) many investigators consider this immunity cell-mediated rather than humoral[6-11]; (2) effective administration of human immune globulin in neonatal herpes of man has not been demonstrated[1,3,12], and (3) transplacental antibody conferred only partial protection in human neonatal infections[1]. There is further discouragement in animal studies which failed to show protection by antibody[9-11], although antibody was protective in some animal studies[13-20]. The findings reported here raise again the possibility that antibody treatment might be effective in specific conditions.

The concept that cell-mediated immunity, rather than humoral immunity, is the major host defence has been based in part on increased severity of HSV infection after impairment of T-lymphocyte function[6-11]. But, findings that HSV antibody production is T-cell dependent[9,20,21] suggest that exacerbation of some of these infections by impaired T-lymphocyte function could have been due to decreased antibody formation[20]. Furthermore, protection of mice that received syngeneic, immune spleen cells and were infected with HSV might, in some cases, be due to the transfer of antibody-producing or helper cells, since high levels of antibody were produced more rapidly in these mice than in control mice (unpublished observations). Consistent with this view is the protection of nude[20], immunosuppressed, and normal mice (unpublished observations) against HSV infection by sufficiently high levels of passively administered antibody and apparent protection against HSV by maternal antibody of infants (excluding newborns) under 11 months of age[22].

Re-examination of antibody potential in a model of neonatal HSV infection is indicated by these findings, even though they do not establish the importance of antibody in all conditions. For example, strains of HSV that spread principally through the blood stream might be affected by antibody, but not strains of HSV that are transmitted through nerve pathways which exclude antibody

Our study was designed to determine whether administration of various units of neutralising antibody could achieve post-exposure prophylaxis of experimental neonatal HSV infection[20]. Mice (Swiss, CDF-1 or BALB/c), age 1–4 d, were infected subcutaneously with from 1 to 300 newborn mouse LD_{50} of HSV, either type 1 (VR3 strain) or type 2 (MS strain). One hour after infection, the experimental mice were injected intraperitoneally with 0.1 or 0.2 ml of type-specific hyperimmune rabbit antiserum. Controls were injected with normal rabbit or foetal bovine serum, or Eagle's tissue culture medium containing 2% foetal bovine serum. Mice were observed for at least 12 d. Percentage protection was calculated as follows:

$$\frac{\%\text{ mortality of controls} - \%\text{ mortality of treated}}{\%\text{ mortality of controls}} \times 100$$

Rabbit antisera were prepared by initial injections of HSV virus either type 1 or type 2 (grown in rabbit kidney cultures and emulsified with Freund's incomplete adjuvant) into the footpads and back (0.25 ml containing $10^{5.4}$ PFUs of HSV), followed by four to eight weekly subcutaneous and intravenous injections of virus without adjuvant.

Protection consistently occurred in newborn mice infected with 1–20 LD_{50} of either type of HSV and treated with a total of 12,000 U of antibody (Table 1). No protection occurred when the large dose of antibody was administered to mice infected with a high challenge dose (100–300 LD_{50}) of HSV. Little or no protection followed treatment with 600 U of antibody. Better results attained against HSV type 1 warrant further study.

Our findings demonstrate good protection against experimental neonatal HSV infection by large doses of antibody in mice infected with low challenge doses of virus. Failure of antibody in some animal studies[9-11], but not in others[13-20], could conceivably be due to such variables as antibody dose[17], virus challenge dose[14], portal of entry HSV, and difference in pathogenesis, depending on the virus strain used. These same factors might account for the reported failures of human immune globulin to protect newborn infants against HSV nfection[1,3,12]. Additional study of these and other variables may further determine the optimum conditions for antibody protection of newborns.

Although our results were obtained using mice infected subcutaneously, other studies have shown antibody protection

Table 1 Protection by antiserum of newborn mice against HSV infection

Expt	Mouse strain (Age, d)	HSV type (strain)	Challenge dose (LD_{50})	Dose of antibody (U per mouse)	Dead mice/ Total mice	% Mortality	% Protection	Combined% protection*
1	CDF-1 (1)	1 (VR3)	10	0	7/7	100		
			10	12,000	1/8	13	87	
2	Swiss (1)	1 (VR3)	2	0	6/9	67		
				12,000	0/10	0	100	
3	Swiss (1)	1 (VR3)	20	0	19/21	90		
				12,000	1/19	5	94	94†
4	CDF-1 (1)	1	100	0	6/7	86		
			100	12,000	6/7	86	0	0
5	BALB/c (1)	2 (MS)	2	0	3/8	38		
				600	5/5	100	0	
			20	0	7/7	100		
				600	7/7	100	0	
6	Swiss (1)	2 (MS)	20	0	18/22	82		
				600	9/22	41	50	18
7	Swiss (4)	2 (MS)	1	0	3/5	60		
				12,000	0/6	0	100	
		2 (MS)	3	0	5/5	100		
				12,000	4/8	50	50	
8	Swiss (2)	2 (MS)	2	0‡	3/5	60		
				12,000	2/8	25	58	
9	Swiss (1)	2 (MS)	3	0	5/5	100		
				12,000	2/6	33	67	
10	Swiss (1–2)	2 (MS)	3	0‡	7/8	88		
				12,000	2/14	14	84	71†
11	Swiss (1–3)	2 (MS)	300	0	18/18	100	0	
			300	12,000	25/25	100	0	0

* Average % protection for preceding experimental group.
† $P < 0.005$
‡ Normal rabbit serum.

of newborn or young animals infected intranasally[13,19], intradermally[13], intracerebrally[14,15,17], intraperitoneally[14], intravaginally[18] or subcutaneously[16]. Because of these earlier findings, human immune globulin treatment in man had been considered[1,12,19,24]. If proper conditions can be defined to protect newborn infants with hyperimmune human globulin, this treatment would offer several advantages over present methods: for example, administration of human immune globulin to newborns is much safer than surgical delivery of the babies or than use of toxic drugs. Finally, combination of protective antibody with other antiviral agents, such as interferon, might enhance protection as with vaccinia virus infection of mice[23].

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Type C virus in lymphosarcoma in northern pike (*Esox lucius*)

THE northern pike (*Esox lucius*) is a highly prized freshwater fish, both as a game fish and as a commerical species. Epizootics of lymphosarcoma occur widely in North American pike[1] and in the Old World[2,3]. The tumour in pike has been found with an overall frequency of 20.9%, which is the highest frequency of a malignant neoplasm in any known free living vertebrate[1]. All pike with the tumour have cutaneous lesions, and epizootiological evidence suggests that the disease is transmitted horizontally by contact during spawning[1]. The tumour is transplantable and evidence of cell-free transmission has been found[1,4]. In an attempt to resolve the aetiology of the disease, we have investigated the presence of oncornavirus in pike lymphoma. Since all known RNA tumour viruses possess the enzyme reverse transcriptase, we have sought the activity of this enzyme in post-mitochondrial particulate fractions prepared from pike lymphoma tissue.

Pike lymphomas were analysed as described in the legend

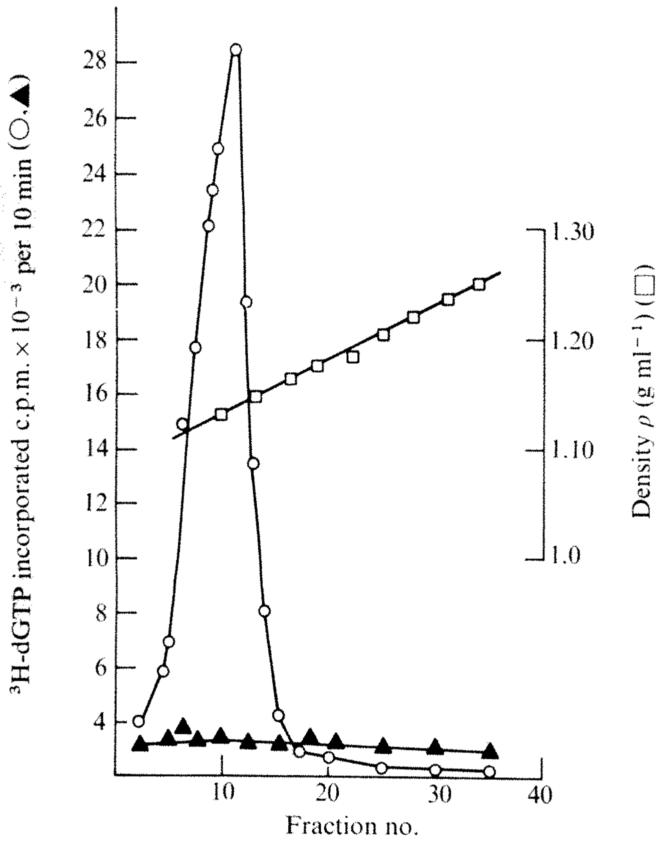


Fig. 1 Identification of the density of reverse transcriptase in a postmitochondrial extract of pike lymphoma by sucrose gradient centrifugation. Frozen pike lymphoma tissue (10 g) was homogenised at 4 °C in 2 volumes of 0.05 M Tris-HCl (*p*H 7.5), 0.01 M dithiothreitol and 0.25 M sucrose. It was then sonicated for 1 min and the suspension was centrifuged at 1,000*g* for 30 min at 4 °C. The supernatant was centrifuged at 16,000*g* for 30 min. The supernatant was again centrifuged at 100,000*g* for 1 h at 4 °C in an SW27 rotor. The resulting pellet (P-100) was suspended in 0.5 ml of 0.01 M Tris-HCl (*p*H 7.5) containing 0.01 M dithiothreitol and layered in a 13 ml 25–60% (w/v) sucrose gradient in TNE and centrifuged at 100,000*g* for 22 h in an SW41 rotor. Approximately 40 fractions were collected from the top by Buchner densiflow. A sample (20 µl) was added in a reaction mixture of (100 µl) containing 100 mM Tris-HCl (*p*H 8.3), 50 mM KCl, 1 mM $MnCl_2$, 4 mM dithiothreitol for poly(Cm).oligo(dG) (20 µg ml^{-1}) and 0.125 M ^{3}H-dGTP. Reaction mixtures were incubated at 20 °C for 1 h and stopped by addition of 10% trichloroacetic acid (TCA). Acid precipitable material was collected on Whatman GF/C filters and the radioactivity was counted. Normal pike tissue was processed in identical conditions and assayed as described above. ○, Pike lymphoma; ▲, normal pike.

to Fig. 1. A cytoplasmic particulate fraction was isolated from the tissue. After further fractionation on sucrose gradients, fractions were assayed for the presence of reverse transcriptase able to utilise poly(Cm)·oligo(dG), a template primer specific for viral polymerases[5]. Reverse transcriptase activity was associated principally with the particulate fraction sedimenting at a density of 1.16 g cm^{-3}. Parallel gradients run with purified Rauscher leukaemia virus were fractionated and assayed similarly for reverse transcriptase activity. Peak activities also occurred at a density of 1.16 g cm^{-3}. When normal pike tissue was analysed identically, no reverse transcriptase activity was detected in any gradient fraction. Thus the reverse transcriptase activity was unique to the lymphoma, and was associated with a particulate fraction corresponding to the density of known RNA tumour viruses.

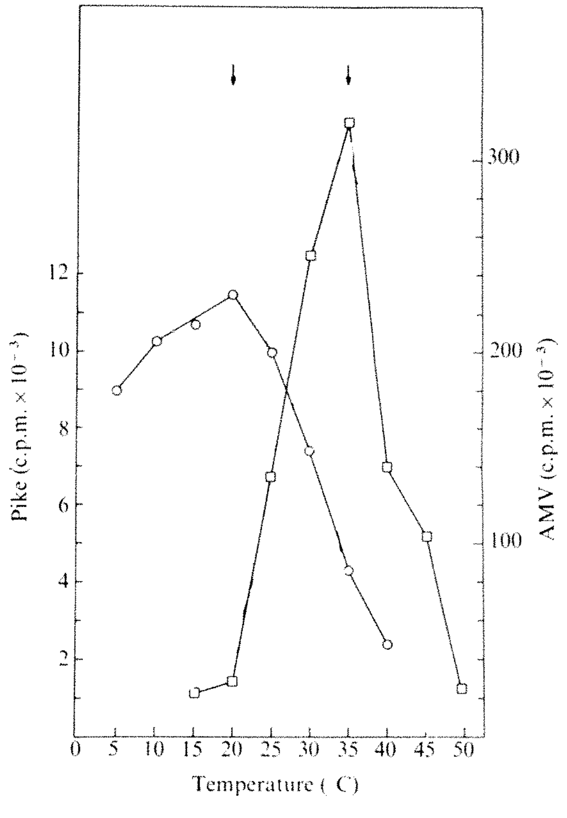
The seasonal nature of the appearance of the pike lymphoma prompted us to examine the temperature profile of the reverse transcriptase activity. As Fig. 2 shows, the enzyme activity from pike lymphoma exhibited 82% of its maximum activity at 5 °C, with an optimum at 20 °C while only 35% was retained at 35 °C. In contrast, reverse transcriptase purified from avian myeloblastosis virus (AMV) has a temperature optimum of 35 °C with only 0.6% of optimal activity at 20 °C and no detectable activity at 5 °C.

Fractions between 1.15 and 1.17 g cm^{-3} were diluted to less than 10% sucrose, pelleted and processed for electron microscopy. Figure 3 shows the appearance of the numerous type C virus-like particles present in these gradient fractions. Most particles appear similar to typical type C virus particles of avian and mammalian origins, but a more careful categorisation must await the detection of budding and immature forms of the virus.

The results reported here demonstrate that pike lymphoma contains poly(Cm)·oligo(dG)-directed DNA polymerase activity present in a structure with the density of an RNA tumour virus. These particles are not disrupted by physical manipulation and maintain their characteristic density when centrifuged repeatedly. This is the first report of the presence of reverse transcriptase in a fish, which is phylogenetically the most primitive animal in which the enzyme has been detected.

The aetiological significance of the type C virus-like particles and its associated reverse transcriptase is unknown. The disease in nature exhibits marked seasonal periodicity, with tumours developing during the cold water periods of fall and winter[1], when mean water temperatures are 12 and 4 °C, respectively. Field epizootiological studies have revealed that the tumours frequently regress spontaneously during the summer months[1], with typical water temperatures varying from 21 to 30 °C. The high activity of the enzyme at low temperatures correlates with the water tem-

Fig. 2 Temperature profiles of partially purified pike and purified AMV reverse transcriptase. Samples were incubated for 30 min in the corresponding reaction mixtures at the temperatures indicated. Reaction mixture temperatures were accurately controlled by using a Lauda circulating water bath. Reactions were stopped by addition of cold 10% TCA, and amounts of ^{3}H-dGMP incorporated into acid-insoluble product was determined. ○, Pike; □, AMV.



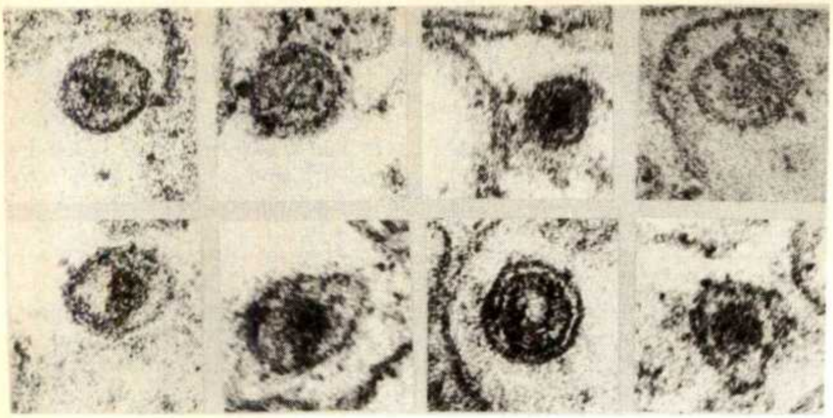


Fig. 3 After frozen tumour had been thawed, homogenised, and fractionated on a sucrose gradient, fractions with densities between 1.5 and 1/17 g cm^{-3} were pooled, diluted and pelleted at 100,000*g*. The pellet was fixed for 2 h in 3% glutaraldehyde in Milling's buffer, washed for 30 s in buffer, post-fixed for 1 h in 1% osmium tetroxide, washed for 2 h in distilled water, stained *en bloc* with 2% uranyl acetate in 50% ethanol, dehydrated in ethanol and propylene acids, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined at 80 kV in a Siemens 101 electron microscope (× 160,000).

peratures at which the tumour develops in nature. The warm water period of summer (non-permissive temperatures) may explain the seasonal periodicity of the disease and the mechanism of the spontaneous regressions observed. The disease in fish offers a unique probe, facilitating both *in vivo* and *in vitro* assays to resolve 'turn on' and control mechanisms of oncornavirus-induced transformation, as both cell cultures and hosts can be placed at permissive and non-permissive temperatures.

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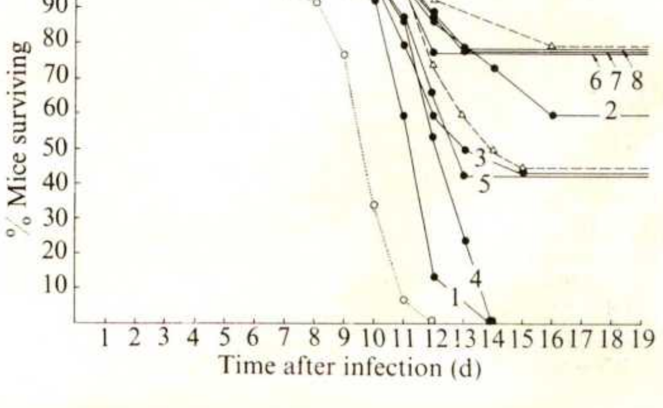
Virulence of different strains of *Toxoplasma gondii* and host response in mice

THE protozoan *Toxoplasma gondii* has a world-wide distribution. It is an obligate intracellular parasite and appears in many species of mammals and birds. Animals that survive the acute stages of *Toxoplasma* infection probably remain infected for life[1]. The RH strain of *Toxoplasma gondii*, originally isolated by Sabin[2] and widely used in the routine Dye test[3] is extremely pathogenic to mice, LD$_{100}$ by intraperitoneal infection being below 10 tachyzoites[4]. Moreover, it is difficult to obtain survival after RH challenge of mice immunised with a strain of lower virulence[5]. Such survival without the use of chemotherapy has, however, been reported[1,6,7]. The RH strain was not totally eradicated[7] from immunised mice even 7 weeks after challenge. How do immunised mice suppress the RH strain—by total elimination or making it possible for the RH strain to remain in the body as does the immunising strain? Furthermore, in the latter case, would the inherent virulence of the RH strain become changed? We here report that *Toxoplasma* possessing the full virulence of the original RH strain, could be isolated from mouse brains 14–18 months after RH challenge of mice which had first been immunised with the Beverley strain. This applied, however, to a proportion of the mice; in the brains of the other mice only the immunising strain (Beverley) was found.

Brain suspensions from test groups (mice infected with the Beverley strain and reinfected with the RH strain) were injected subcutaneously into normal mice (Fig. 1). In two of the test groups (1 and 4) all mice died as did the RH controls. In the others a varying proportion of the mice succumbed, an event which also occurred in Beverley infected controls. In this comparison the RH control infection started from the tachyzoite stage, whereas the others started from a cystic stage. To establish a comparison with all infections arising from the tachyzoite stage, the following procedure was undertaken : Two mice from each of the test groups 1, 2 and 3 as well as the RH and Beverley controls were killed after 6–9 d, brain suspensions were made and injected into groups of normal mice (Fig. 2). In test groups 2 and 3 all mice survived as did the Beverley controls. The surviving mice had become Dye test-positive, proving that they had gone through a *Toxoplasma* infection. In test group 1 all mice died, the survival time being almost identical to that found for RH-infected mice, indi-

Fig. 1 Survival rates of mice after infection with *Toxoplasma gondii*. Brain suspensions of mice (female NMRI/Bom) which had been infected with *Toxoplasma gondii* were injected into normal mice. Brains were derived from mice which had been infected in three different ways. ●, Test groups. Mice which had been infected first with the Beverley strain and then with the RH strain (5 × 10^2 or 5 × 10^5 tachyzoites intraperitoneally) 2 weeks–4 months later. 14–18 months after the RH challenge the brains were dissected out. Number of the individual test group as shown. △, Mice which had been infected merely with the Beverley strain 12 months previously. ○, Mice with a RH infection of 4 d duration. Brains were mortared in 0.9% phosphate-buffered saline. Each brain suspension to be tested was prepared from one mouse or from two parallel mice. The brain suspensions were diluted so that one brain would provide enough material for infection of 20 new mice. Aliquots (0.3 ml) of the brain suspensions were injected subcutaneously to each normal mouse (9–20 mice in each group).



cating that we were dealing with the RH strain in test group 1, but not in test groups 2 and 3.

Further support was obtained from experiments in which the brain suspensions from test groups and controls were examined with regard to their ability to produce peritoneal exudate. The virulent RH strain is much more efficient than the Beverley strain in this respect. In test groups 1 and 4 a large amount of peritoneal exudate (about 1 ml) was found in all five mice tested. In test group 5 two out of five mice showed this marked exudate production. In the other test groups only small amounts of exudate (about 0.1 ml) were found. A direct comparison of the virulence of the tachyzoites isolated in the different groups was then carried out By dilution a low dose (5×10^2 tachyzoites per ml) was prepared in each group and aliquots of 1 ml of these suspensions were injected intraperitoneally to normal mice (Table 1). Tachyzoites of test groups 1, 4 and 5 were as efficient in producing peritoneal exudate as the RH strain.

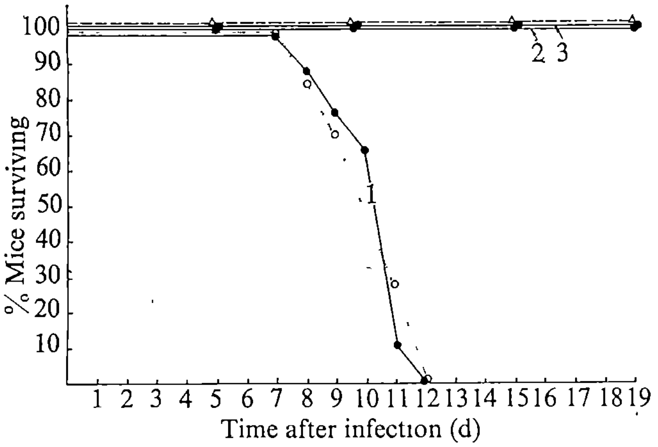


Fig. 2 Survival of mice after infection with *Toxoplasma* tachyzoites. Two of the mice in each group infected with brain suspensions from test groups 1, 2, 3 and the controls (see Fig. 1) were killed after 6–9 d Brains were mortared in saline and diluted as described in Fig. 1. These brain suspensions were injected into groups of normal mice (7–15 mice in each group). Symbols as in Fig. 1.

Table 1 Virulence of isolated *Toxoplasma gondii*

Donor mice	Recipient mice: Production of peritoneal exudate: Volume (ml) Mean ±1.s.e	No. of extracellular tachyzoites (per mouse) Mean ±1 s.e	Survival time (d) Mean (range)
Test group No. 1	1.0±0 1	$28\pm4\times10^6$	7 4 (7 0–8 0)
4	0.9±0.1	$19\pm3\times10^6$	7.8 (7.5–8.0)
5	1.2±0.1	$24\pm3\times10^6$	7.6 (7 0–8.5)
6	0.1	$18\pm5\times10^3$	All survived
7	0.1	$14\pm9\times10^3$	All survived
8	0.1	$10\pm3\times10^3$	All survived
RH control	0.9±0.1	$29\pm7\times10^6$	7.1 (6.5–8.0)
Beverley control	0.1	$10\pm4\times10^3$	All survived

The peritoneal exudates isolated from test groups and from controls were centrifuged at 65*g* for 5 min to sediment the cells. The supernatants containing the tachyzoites were diluted in phosphate-buffered saline (*p*H 7.2) to give 5×10^2 tachyzoites ml^{-1} Aliquots (1 ml) of these suspensions were injected intraperitoneally to normal mice (that is, recipient mice). Five mice in each group were used to evaluate survival times and five other mice to evaluate production of peritoneal exudate. The latter mice were killed after 5 d infection, the volumes of the peritoneal exudate which could be aspirated were measured, and the numbers of extracellular tachyzoites in the exudates were recorded by counting in a haemocytometer

Furthermore, in these groups all mice tested for survival died. In the other test groups all mice survived and only small amounts of peritoneal exudate were found. The surviving mice had all produced antibodies against *Toxoplasma*, as demonstrated by the Dye test. Thus *Toxoplasma* isolated from test groups 1, 4 and 5 behaved exactly like the RH strain.

From these experiments the following conclusions can be drawn. Mice surviving a Beverley infection continue to carry the parasite in their brains. These mice have the ability to survive a consecutive RH infection. In such double-infected mice the RH strain can be found in the brains of some of the mice 1.0–1.5 yr later. Once outside the brains, the strain is as virulent as the original, routinely transferred RH strain. Even though we did not find the RH strain in the brains of the major proportion of the mice, the possibility still exists that it could be present at other sites, since *Toxoplasma* has been demonstrated to form cysts in a number of organs[8,9].

From this model, which could be expected to have relevance for other mammals, some interesting aspects arise A person infected with a strain of low virulence and later with a more virulent strain, may benefit immunologically from the first infection in the extent to which acute disease may be prevented. If the virulent strain remains in the body and keeps its inherent virulent potentiality, however, such a person might under certain circumstances be more threatened than if he had been harbouring a strain of low virulence. First, the possibility exists that the virulent strain could evoke disease from organs not well protected by the immune apparatus—for example, chorioretinitis. Second, during immunosuppressive therapy or debilitating disease a virulent strain might be more likely to be reactivated than a strain of lower virulence.

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Rates of oxygenation and Bohr shift of capillary blood in chick embryos

THE effect of carbon dioxide on the affinity of red blood cells (RBC) for oxygen was first described by Bohr, Hasselbalch and Krogh[1] The higher the P_{CO_2} and/or the lower the *p*H, the lower the O_2 affinity. The rate of the affinity change, that is, the Bohr shift, has been measured by several investigators[2–4] by abruptly changing the P_{CO_2} of a suspension of RBC using a rapid flow reaction apparatus combined with a P_{O_2} electrode. They showed that the Bohr shift was much slower than the simple oxygenation and deoxygenation rates. While developing a microphotometer technique for kinetic studies of a single RBC in the presence of O_2 and CO (refs 5 to 8), we also attempted to determine the rate of the Bohr shift in the capillary blood of chick embryos, and found that the Bohr shift rate was slower than the O_2 reaction rates; in particular, in the cooperative reactions of O_2 and Bohr shift, the O_2 reaction appeared first followed by the Bohr shift

Figure 1 represents the O_2 dissociation curves of 16-d-old

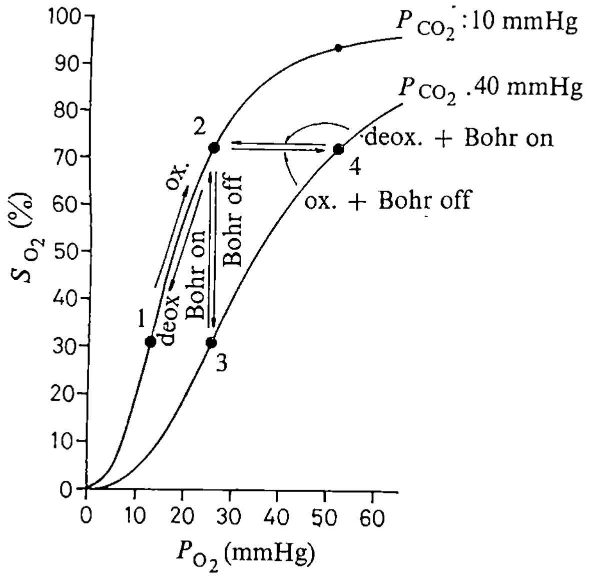

Fig. 1 The oxygen dissociation curves for the capillary blood of 16-d-old embryos determined with the microphotometric apparatus. The closed circles on the curves indicate the reaction gas mixtures used. Gas mixture 1, 13 mmHg P_{O_2} and 10 mmHg P_{CO_2}; 2, 26 mmHg P_{O_2} and 10 mmHg P_{CO_2}; 3, 26 mmHg P_{O_2} and 40 mmHg P_{CO_2}; and 4, 52 mmHg P_{O_2} and 40 mmHg P_{CO_2}. The changes of gas mixtures are shown by arrows.

chick embryos[9], where P_{O_2} at 50% O_2 saturation (P_{50}) is 17.5 and 35 mmHg for 10 and 40 mmHg P_{CO_2}, respectively. As the P_{CO_2} is decreased from 40 to 10 mmHg, the curve of 40 mmHg P_{CO_2} shifts to the left. Thus, when the P_{O_2} is kept constant, for example at 26 mmHg, the O_2 saturation (S_{O_2}) increases from point 3 to point 2 on the curves in Fig. 1. On the other hand, when the P_{CO_2} is kept constant at 10 mmHg and P_{O_2} is increased from 13 to 26 mmHg, oxygenation occurs and the S_{O_2} increases from point 1 to point 2. Now, if the O_2 diffusion is assumed to be fairly slow compared with the shift of the O_2 dissociation curve, the increase of S_{O_2} in the above two cases should occur at the same rate, because the S_{O_2} values of points 1 and 3 are identical and the P_{O_2} gradients driving the oxygenation are equal to each other. Moreover, when the P_{O_2} and P_{CO_2} are varied simultaneously from 13 to 26 mmHg and from 10 to 40 mmHg respectively (or from 26 to 52 mmHg and from 10 to 40 mmHg respectively), no change in S_{O_2} should occur.

To clarify these speculations we attempted to measure the rate of the S_{O_2} change of RBCs in the chorioallantoic membrane of 16-d-old chick embryos. The membrane forms a single layer capillary plexus of 6 to 10 μm thickness which is a gas exchange organ of the embryo[10,11]. The change in S_{O_2} of a single cell in the capillary was measured through the membrane with a microphotometer[5,6]. The membrane was placed in a small air-tight reaction cuvette and the compositions of O_2 and CO_2 around RBCs were changed stepwise by flushing gas mixtures through the cuvette one after another. The gas mixtures used are numbered on the curves in Fig. 1. The additional gas mixtures 0 and 5 have 0 mmHg P_{O_2} and 300

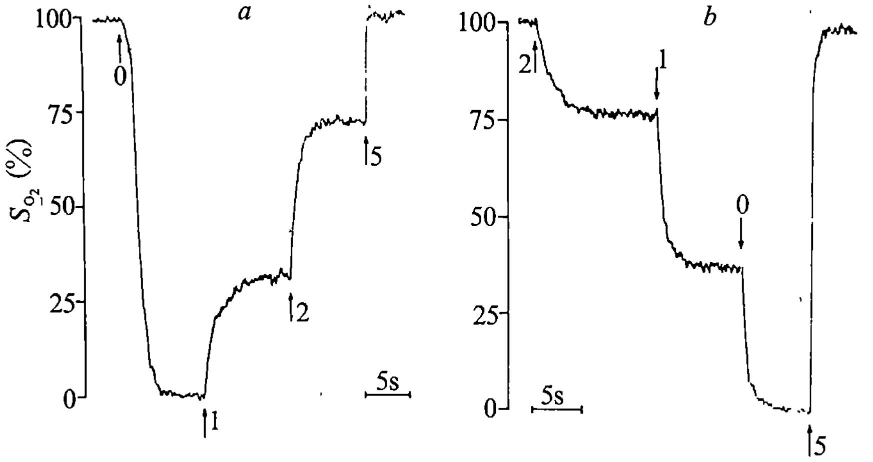

Fig. 2 The O_2 reaction of capillary blood. *a*, Oxygenation reaction The blood was first deoxygenated completely by flowing the O_2-free gas mixture (0). The colour change of the RBCs which accompanies the O_2 reaction was detected by a couple of photomultipliers with reference filters of 402 and 418 nm wavelengths[5,6] After equilibration, the second gas mixture (1) was flushed through. The oxygenation reaction was then recorded by flowing the third gas mixture (2), and the fourth gas mixture (5) was finally used to attain 100% S_{O_2} state. *b*, Deoxygenation reaction.

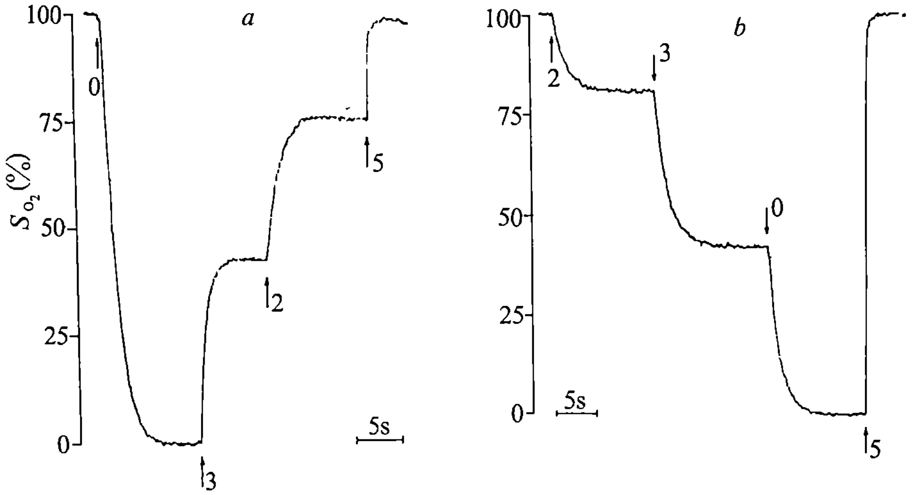

Fig. 3 The Bohr shift reaction. *a*, The Bohr on-shift reaction made by a sudden change in P_{CO_2} from 40 to 10 mmHg keeping P_{O_2} constant (26 mmHg). *b*, The Bohr off-shift reaction.

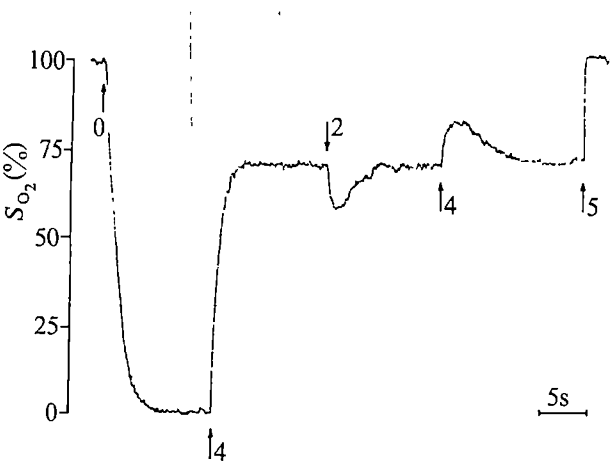

Fig. 4 The cooperative reaction of O_2 and CO_2. After complete deoxygenation, the blood was equilibrated with gas mixture 4. In the cooperative reaction of O_2 and CO_2 induced by a sudden change from gas mixtures 4 to 2, the deoxygenation due to the P_{O_2} decrease from 52 to 26 mmHg appears first and then the Bohr on-shift reaction recovers the S_{O_2} to the initial level at a slower rate The opposite reaction, first oxygenation and second Bohr off-shift, was observed by flushing gas mixture 4 after regaining the equilibrium with gas mixture 2.

mmHg P_{O_2} to provide 0% and 100% S_{O_2} level, respectively.

The changes of S_{O_2} in the simple oxygenation and deoxygenation reactions are seen in Fig. 2*a* and *b*, respectively. The arrow in the recorded pattern indicates the time of the flush of a gas mixture. Precise analysis with a high speed response oscillograph showed that the half-time was about 0.4 s for both cases. The changes of S_{O_2} in the Bohr on- and off-shifts are shown in Fig. 3*a* and *b*, respectively. The S_{O_2} change was a simple exponential in every case The rates of the Bohr shift reaction, however, were slower than those of the O_2 reactions shown in Fig. 2; the half-time was about 0.9 s for both shifts. Then, the cooperative kinetic reactions of O_2 and Bohr shift were performed in such a manner that the P_{O_2} around RBCs was varied together with the P_{CO_2} so as to make equal the S_{O_2} before and after the reaction (Fig. 4). If the O_2 affinity were to shift very rapidly as the P_{CO_2} was changed, the S_{O_2} change should not occur under such conditions that the P_{O_2} outside RBC was varied at the same rate as P_{CO_2} and as much as the O_2 backpressure. The S_{O_2} change appears as shown in Fig. 4, however, that is, the O_2 reactions appear first, and change the S_{O_2}, and the Bohr shift reaction follows to return the S_{O_2} level to its initial state. In another experiment, we found that the Bohr shift did not occur when activity of carbonic anhydrase was inhibited by acetazolamide. Additionally, the CO_2 diffusion into RBC is estimated to be 20 times as fast as the O_2 diffusion. Therefore, the rate of the O_2 affinity change due to the CO_2 may be retarded by the rate at which P_{CO_2} and *p*H come into equilibrium or the reaction rate of H^+ and haemoglobin. As a result, the Bohr shift might become slower than the oxygenation and deoxygenation reactions. At any rate, it seems certain that the Bohr effect is noticeably small in such a short time as the 1 s or so of the transit time through the gas exchange area.

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Correlation between biological potency and biodegradation of a somatostatin analogue

ONE approach to the problem of producing peptide hormones of high activity for clinical application is to synthesise analogues which are less rapidly inactivated[1]. In the case of luliberin, it was found that an analogue with an inserted D-amino acid and a blocked C terminal end was 80 times more active than the native peptide *in vivo*. This correlated well with the decreased lysis of the analogue by neutral endopeptidases[2]. There is at present considerable interest in utilising somatostatin clinically. This use is drastically limited by its very short half life of less than 5 min (ref. 3) Somatostatin inhibits the release of growth hormone, thyrotropin and prolactin from the pituitary, of glucagon and insulin from the pancreas, and also of gastrin[3–5]. As a consequence there is considerable incentive to prepare longer acting agonists for use in diabetes, and for treatment of developmental disorders.

In previous studies we established that two mechanisms are involved in the breakdown of somatostatin in rat brain: release of N-terminal Ala and Gly by an aminopeptidase, and cleavage at an internal site involving the aromatic residues Trp and Phe (Phe–Phe, Trp–Lys, Thr–Phe) (ref. 6; Fig. 1). Based on the sequential release of amino acids with time, the -Trp⁸–Lys⁹ bond seemed to be the most vulnerable Early studies showed that aminopeptidase action is limited, and that carboxypeptidase activity is not a significant factor in breakdown. The only analogues available for the earlier study were *N*-blocked, such as *N*-acetyl-des-Ala¹-Gly²-somatostatin, but biological tests in man and animals have been disappointing[7,8]. This may be explained by the failure to block endopeptidase activity at internal sites. In the present study, we have used another analogue containing a D-Trp in position 8 which is known to have an improved biological potency[9]. It is thus of considerable interest to determine whether the increased activity arises because of blocking an endopeptidase at the critical Trp⁸–Lys⁹ peptide bond (Fig. 1).

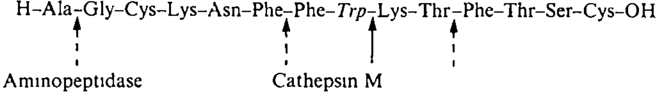
S
S
H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-*Trp*-Lys-Thr-Phe-Thr-Ser-Cys-OH
Aminopeptidase
Cathepsin M

Fig. 1 Proposed primary (↑) and secondary (⇡) sites for cleavage of cyclic somatostatin by a brain neutral endopeptidase (cathepsin M) Replacement of Trp in position 8 by D-Trp blocks cleavage of the D-Trp-Lys bond by the endopeptidase

For studies on breakdown of (D-Trp⁸)-somatostatin in blood, and in rat brain homogenates, the strategy adopted was the same as that applied to luliberin, substance P, and kinin-9 (refs 2, 10 and 11). The method is simple in principle and is based on the timed release of amino acid constituents measured with the aid of an autoanalyser as described previously[12]. In a crude mixture of enzymes some cleavage sites are rate limiting and the order of amino acid release provides information on the peptide bonds involved. This approach has been validated by the use of analogues with blocked cleavage sites, by the isolation of some of the intermediate peptide products and by the use of suitable bioassay procedures[2,13,14]. This method is particularly useful when the nature of the inactivating enzyme is unknown, since it does not require preliminary purification of the enzyme nor the isolation of complex intermediate breakdown products.

Results for the critical residues adjacent to the sensitive bonds

Table 1 Cleavage of somatostatin and the D-Trp^8 analogue by rat brain homogenate and rat serum

Substrate	Time (h)			nmol %			
		Ala^{1*}	Gly^2	Lys^{4,9}	Trp^8	D-Trp^8	Phe^{6,7,11}
Brain							
Somatostatin	4	17	37	45	40	—	37
D-Trp^8-somatostatin	1	0	0	0	0	—	17
	4	24	26	0	—	0	28
Serum							
Somatostatin	4	44	50	58	60	—	46
D-Trp^8-somatostatin	4	50	60	Trace	—	Trace	0

*Numbers refer to the position of residues adjacent to bonds sensitive to proteolytic cleavage in Fig. 1.

Trace, < 5 %

Incubation mixture consisted of 50 nmol somatostatin or its D-Trp analogue in 0.2 ml Tris-HCl 10 mM, *p*H 7 6, containing 0.1 mM Cleland's reagent to which was added 0 2 ml 10% rat brain homogenate or 0.2 ml rat serum. Incubations were for 1–4 h at 37 °C and the reaction terminated by the addition of an equal volume of 6% w/v sulphosalicylic acid. Breakdown products in the supernatant fraction were assayed with the aid of an autoanalyser as described previously[12].

Potency *in vitro* (release of immunoassayable growth hormone) was 848 (confidence limits of 95%, 518–1416) compared with somatostatin at 100; *in vivo* (release of glucagon and insulin) was 821 (368–2195) compared with somatostatin at 100 Data provided by J. Rivier, Salk Institute, San Diego, California.

for the aminopeptidase and endopeptidase show a complete blockage of D-Trp^8 and Lys^9 release for the analogue when incubated with brain homogenate and serum (Table 1) In rat brain, the release of these two residues was reduced from 40–45 nmol % to zero, and release of Phe was reduced from 37 to 28 nmol %. In terms of the aminopeptidase action, release of Gly was reduced from 37 to 26 nmol %. With rat serum, the reduction in release of Phe was even more striking since no Phe was detected even after 4 h at 37 °C. In terms of biological activity, Rivier and his associates have shown that there is a six- to eightfold increase in the ability to inhibit the release of growth hormone *in vitro* and glucagon and insulin *in vivo*[9].

These results are therefore consistent with the hypothesis that biological activity can be correlated with a change in biodegradation. It is therefore evident that in the case of brain the introduction of D-Trp in position 8 did not completely block the release of Phe (in positions 6, 7 or 11); this may account for an increase in potency but not necessarily for a longer duration of action. It would be necessary to block all internal sites to ensure a completely stable peptide in terms of neutral endopeptidase activity There were some notable differences between cleavage of (D-Trp^8)-somatostatin by brain and serum enzymes since the release of Phe was not observed with the latter This may mean that (D-Trp^8)-somatostatin is more stable during transport in serum but once it reaches its target site it can be inactivated at the secondary Phe^6–Phe^7 or Thr^{10}–Phe^{11} sites (Fig. 1). Since a larger proportion of the peptide would then be expected to reach the target site before destruction this could account for enhanced activity without a longer duration of action.

This explanation of the improved biological potency of the (D-Trp^8)-somatostatin analogue does not exclude the possibility of an improved binding by the analogue to the relevant receptors. Studies on the binding of (D-Trp^8)-somatostatin are unavailable, but in the case of other hormonal releasing factors there is often a remarkable correlation between increased activity and binding[15]. An improvement in receptor occupancy however does not necessarily ensure a longer duration of action and this may depend on a combination of factors, one of which is resistance to enzymic degradation.

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Covalently closed circular DNA molecules of low superhelix density as intermediate forms in plasmid replication

COVALENTLY closed circular (CCC) duplex extrachromosomal DNAs are widespread in biological systems[1]. Because of their physical properties and small size, extra chromosomal circular DNAs are useful for studying the biology of genetic material. Their mechanism of replication is of particular interest and has been investigated extensively. Recent studies have shown that the replication of different types of circular DNA, such as plasmids of *Escherichia coli*, the DNA of polyoma and SV40 viruses, and mitochondrial DNA is basically similar[2,3]. Non-replicating circular DNA is isolated from cells largely as covalently closed supercoiled molecules (form I DNA). Because of its topological constraints, CCC DNA has a restricted affinity for intercalating dyes such as ethidium bromide and propidium diiodide; its buoyant density in gradients of CsCl containing such dyes is consequently much greater than that of non-circular or nicked circular (form II) DNA species, which can unwind to accommodate maximal amounts of dye[4]. DNA molecules in the process of replication have been shown to have buoyant densities intermediate to form I and form II DNA (refs 5–12 and L. Katz, P. H. Williams, S Sato, R. W. Leavitt, and D R Helinski, unpublished). In a previous study of replicative forms of a constructed recombinant plasmid (pSC134) of *E. coli*[13], we observed material pulse labelled with radioactive thymidine that had a buoyant density in CsCl–ethidium bromide gradients even greater than that of form I DNA[5]. Here we report results which suggest that this material, termed "heavy replicative DNA" or hrDNA, is a covalently closed circular DNA species that has a reduced number of supercoils, and is an intermediate in the replicative process.

Figure 1 shows the kinetics of appearance and disappearance

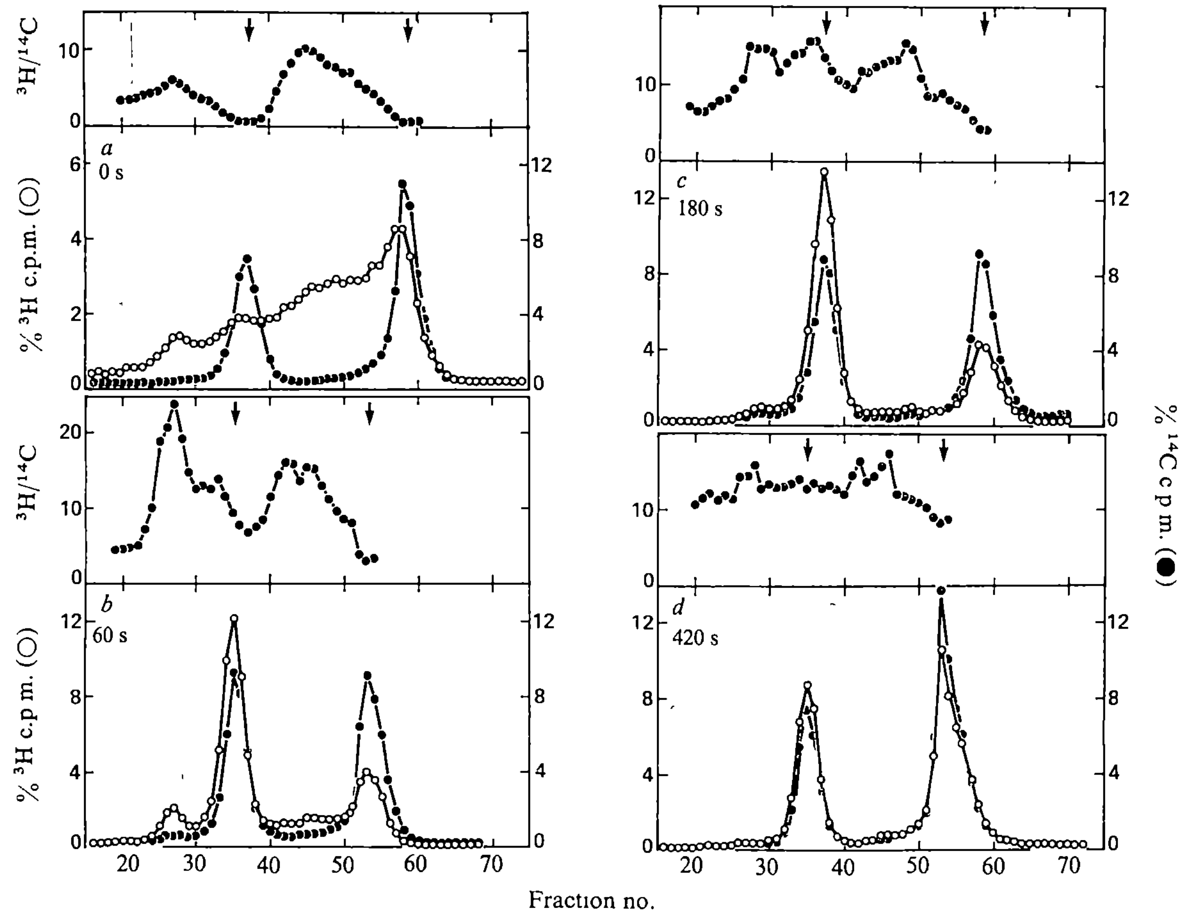


Fig. 1 Caesium chloride–propidium diiodide equilibrium centrifugation analysis of pulse–chase DNA from pSC101-carrying bacteria. 100 ml cultures of *E. coli* K12 CR34 Nal^r (pSC101)[5,32] in M9 medium containing 0.5% casamino acids, 10 μg ml^{-1} thiamine, 3 μg ml^{-1} thymidine and 0.1 μCi ml^{-1} ^{14}C-thymidine were grown with aeration at 37 °C to an A_{590} of 0.4, resuspended in fresh, prewarmed medium without thymidine, and incubated for 30 min at 37 °C. The thymidine-starved cultures were cooled to 20 °C, pulse-labelled for 20 s at this temperature by addition of thymidine to 0.1 μg ml^{-1} and ^{3}H-thymidine to 10 μCi ml^{-1} and then chased for various periods of time, (0, 60, 180, 420 s; panels *a*, *b*, *c*, *d*, respectively) by addition of non-radioactive thymidine to 500 μg ml^{-1}. Pulse–chases were terminated by pouring the cultures into 1,000-ml flasks which were precooled in dry-ice–isopropanol baths and which contained equal volumes of ice–KCN (final concentration 20 mM). Bacteria were washed twice with 100 ml volumes of ice-cold TE buffer (10 mM Tris-HCl, *p*H 8.0–1 mM EDTA) containing 20 mM KCN, lysed with Triton X-100 and "cleared lysates" prepared[1]. The cleared lysates (8.5 ml volumes) were mixed with CsCl (8.0 g) and propidium diiodide (280 μg ml^{-1}) and centrifuged at 36,000 r.p.m. for 60 h at 20 °C in a Beckman 50 Ti rotor. Fractions were collected from the bottom of centrifuge tubes and the cold trichloroacetic acid, precipitable ^{3}H and ^{14}C radioactivity present in a 25-μl sample of each fraction was determined. In the figures, density increases from right to left. The upper part of each main panel shows the distribution of ^{3}H/^{14}C ratios. Arrows indicate the positions of non-replicating, covalently closed circular form I plasmid DNA (left) and nicked circular form II plasmid and non-circular DNA (right). Note that the magnitude of the ^{3}H scale for the 0 s chase in one-half that of the other chases.

of pulse-labelled hrDNA of the pSC101 plasmid. In this experiment, the total DNA of thymidine-requiring bacteria containing pSC101 was prelabelled with ^{14}C-thymidine and the cells were washed and then starved of thymidine for 30 min. Replicating DNA was pulse labelled by the addition of ^{3}H-thymidine, and the radioactive label was chased with cold thymidine for various periods of time. Extracts ("cleared lysates") of bacteria were centrifuged to equilibrium in dye–CsCl gradients; in this instance we used the dye propidium diiodide to increase resolution of various DNA species[14]. Most of the DNA labelled during a 20-s pulse of ^{3}H-thymidine bands at a position between form I and form II DNA (Fig. 1*a*). Some of the pulse-labelled material is, however, seen to band in the gradient at a buoyant density greater than that of form I DNA. This is hrDNA.

After a 60-s chase with unlabelled thymidine, much of the radioactivity incorporated into the various pulse-labelled DNA species is found at the position of form I DNA; however, although the amount of labelled material present in the intermediate density peak is diminished, the amount of heavy material is increased (Fig. 1*b*). Thus, formation of hrDNA correlates with the appearance of pulse label in mature supercoiled DNA and with the disappearance of replicative intermediates of intermediate density. With chases lasting more than 60 s, the amount of heavy material progressively decreases until it has disappeared by 420 s (Fig. 1*c* and *d*). The observed kinetics of appearance and disappearance of pulse-labelled heavy material suggest that it represents a plasmid replicative intermediate; it appears transiently during plasmid replication, and is rapidly converted into form I DNA during a chase with unlabelled thymidine.

hrDNA is also heterogeneous in buoyant density (Fig. 1) and seems to contain two major components that band 9 and 4 fractions, respectively, ahead of form I DNA in conditions which separate form I and form II DNA by 19 fractions (Fig. 1*b*). During the chase period, the amount of the heavier component of hrDNA decreases from 7% of the total ^{3}H-c.p.m. pulse label (60-s chase) to 2% (180-s chase) to 1% (420-s chase), but this component does not change position in the gradient. In contrast, during the same period, the lighter component of hrDNA moves progressively from a position four fractions ahead of form I DNA to a position of equal density to this DNA.

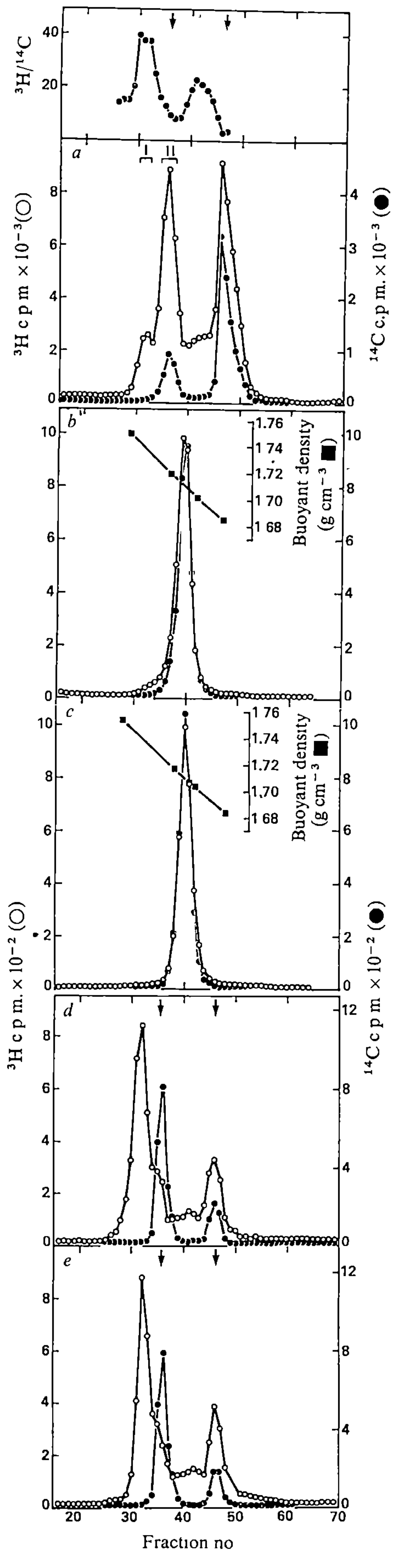
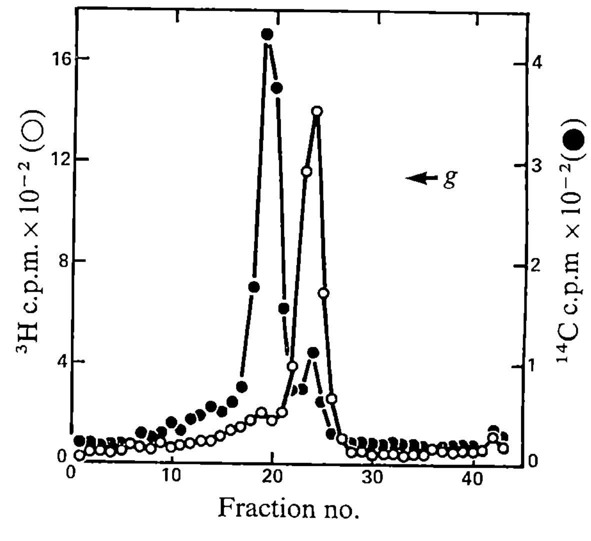


Fig. 2 Ribonuclease A digestion of heavy replicative pSC101 DNA A 250-ml culture of *E coli* K12 CR34 Nal^r (pSC101) was pulse labelled with ^{3}H-thymidine (20 μCi ml^{-1}) and chased for 60 s with cold thymidine, as described for Fig 1. A cleared lysate was prepared and 8 ml mixed with 8 g of CsCl plus 1 ml ethidium bromide (3 mg ml^{-1}) and was centrifuged to equilibrium (36,000 r.p.m. for 60 h at 20 °C). Panel *a* shows the distribution of ^{3}H and ^{14}C radioactivity in this gradient. The dense replicative DNA obtained (pool I) was extracted twice with isopropanol equilibrated with TE buffer saturated with CsCl to remove ethidium bromide It was subsequently dialysed for 3 h against 1,000 ml TE buffer (two changes), and samples treated (*c* and *e*) or not (*b* and *d*) with RNase A (Worthington) in low salt buffer, as described by Blair *et al*[19]. After removal of the RNase by digestion with Pronase[19], the DNA samples were mixed with ^{14}C-labelled reference pSC101 form I DNA and analysed by centrifugation to equilibrium in CsCl (*b* and *c*) or CsCl–ethidium bromide (*d* and *e*). The buoyant densities of selected fractions from the CsCl gradients were determined by measurement of their refractive indices. In *d* and *e* it can be seen that some nicking of CCC DNA had occurred after isolation but before RNase treatment. The proportion of nicked DNA was not increased by RNase treatment

The position of hrDNA in dye–CsCl gradients could result from the presence of substantial amounts of RNA in the DNA molecules or from a decrease in their superhelix density[15]. To investigate the possible presence of RNA we prepared some of the heavier component of hr-pSC101 DNA (pool I of Fig. 2*a*) and analysed it by equilibrium centrifugation in CsCl gradients before or after treatment with RNase A at low salt concentration (Fig. 2*b* and *c*). An observed buoyant density shift in CsCl (0.001 g cm^{-3}) of ^{3}H-labelled hrDNA after RNase treatment to a position coincident with ^{14}C non-replicating pSC101 DNA showed the presence of an RNA component (calculated to represent about 1% of the DNA (ref. 16)) within hrDNA.

Centrifugation of RNase-treated heavy replicative material in CsCl–ethidium bromide gradients showed no detectable shift in buoyant density occurred following RNase treatment (Fig. 2*d* and *e*), indicating that the RNA shown to be present in hrDNA does not account for its banding properties in dye–CsCl gradients. Moreover, these data indicate that the RNA component of hrDNA is not covalently linked to the CCC DNA

Fig. 3 Velocity sedimentation analysis of heavy replicative pSC101 DNA A sample of the dense material from pool I of Fig. 2*a* was dialysed for 3 h against 1,000 ml TE containing 1 M NaCl (two changes), mixed with ^{14}C-labelled reference form I pSC101 DNA and centrifuged through a 5 2-ml 5–20% neutral sucrose gradient at 40,000 r p.m for 180 min at 20 °C in a Beckman SW50.1 rotor. Fractions were collected on filters which were subsequently dried, washed with cold trichloroacetic acid and counted in a liquid scintillation counter.



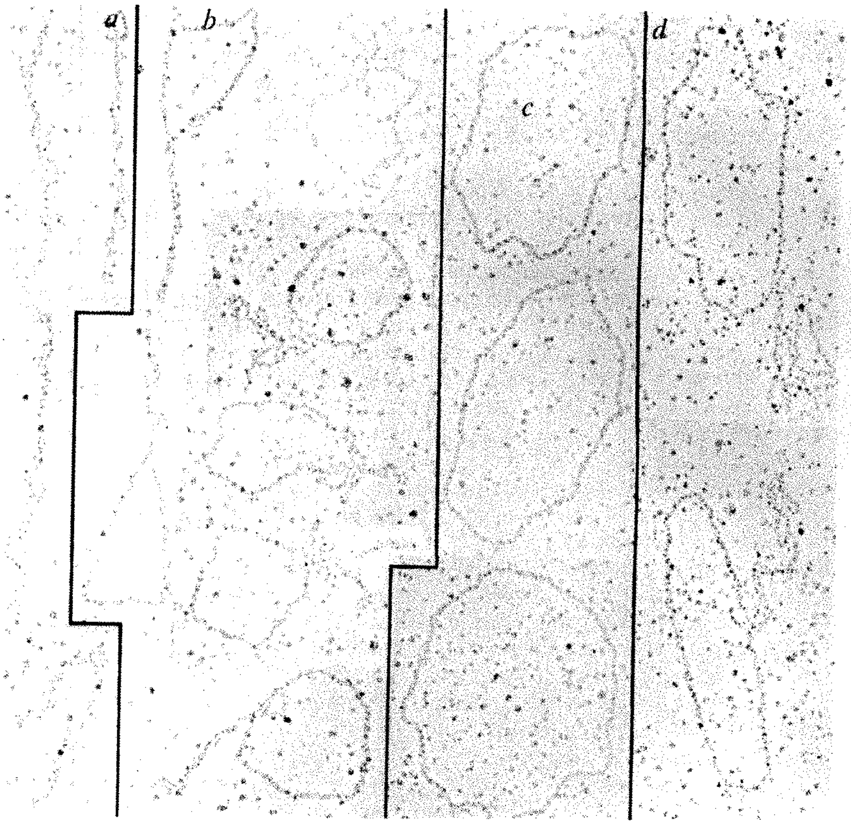


Fig. 4 Electron microscopy of heavy replicative pSC101 DNA. A sample of pool I from Fig. 2*a* was treated with isopropanol to remove ethidium bromide, dialysed against TE buffer and mounted for electron microscopy by means of the basic protein film method[33,34]. Four main molecular forms of DNA were observed: highly supercoiled (form I) molecules (*a*); molecules with fewer superhelical turns, often with a partially open configuration (*b*); molecules with no supercoils (*c*); and catenated dimers consisting of one supercoiled monomer interlocked with a second open monomer (*d*; because these catenated dimers have buoyant densities equal to, or greater than, form I DNA, we conclude that the component of the catenate that lacks supercoils is nevertheless covalently closed). The relative number of each species present in this preparation are (for comparison, the relative number of similar forms present in the CCC DNA region of the same gradient, that is, pool II of Fig. 2*a*, are given in parentheses): *a*, 30 (134); *b*, 10 (1); *c*, 151 (3); *d*, 4 (4). The above photographs and numbers are derived from molecules present on several adjacent squares of a single grid. The reason for most molecules with a reduced superhelix density adopting a form with a terminal loop (*b*) is not known.

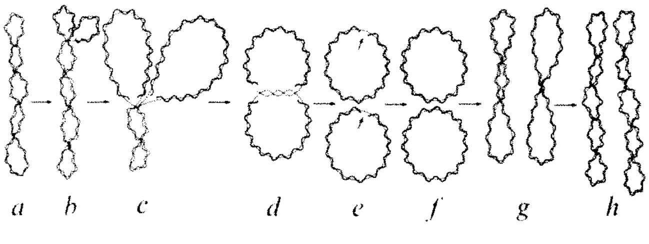
component, since RNase digestion does not produce nicking of this material (that is, conversion to a form II species). It remains to be determined whether the RNase-sensitive material associated with hrDNA is contaminating RNA or is RNA that has a plasmid-related function, such as the priming of DNA synthesis, as has been shown with ColE1 (refs 17–19).

Analysis of hrDNA by neutral sucrose centrifugation also supports the view that it consists of CCC molecules with a reduced number of supercoils. Although its buoyant density characteristics in dye–CsCl gradients indicate that hrDNA consists of covalently closed DNA circles, the heavier component of hrDNA sediments with, or slightly ahead of, form II or nicked pSC101 DNA in 5–20% neutral sucrose gradients (Fig. 3), as expected for a monomeric DNA species lacking superhelical turns[20]. The light components of hrDNA have greater sedimentation velocities (our unpublished data), consistent with an increasing number of twists as the DNA species approaches the buoyant density and superhelix density of form I DNA (ref. 20).

Because one might be able to demonstrate directly the postulated paucity of supercoils in the heavy material, we examined hrDNA (pool I of Fig. 2*a*) in the electron microscope and compared it with non-replicating form I DNA (pool II of Fig. 2*a*). Whereas most of the molecules from the form I region of a CsCl–ethidium bromide gradient are highly supercoiled (134 of 142; Fig. 4*a*), most of those present in pool I hrDNA are completely untwisted (151 of 195; Fig. 4*c*) and have the same appearance as nicked pSC101 circular monomers. Furthermore, some molecules that have a reduced superhelix density and seem to be partially open and partially supercoiled (10 of 195; Fig. 4*b*) are present in pool I DNA but are not present in significant numbers (1 of 142) in pool II DNA. Such molecules probably represent those present in the light component of hrDNA which is seen as a shoulder of form I DNA in dye–CsCl gradients (Figs 1 and 2). The types of molecules present in pool I DNA are thus consistent with the interpretation that hrDNA is composed of CCC molecules with reduced superhelix densities. The absence of obvious replication "eyes' or forks in hrDNA is consistent with our inability to label this material with very brief (5 s) ^{3}H-thymidine pulses in the absence of a chase (our unpublished data) and suggests an absence of nascent DNA. Thus, although these data do not exclude a role for hrDNA in the initiation of replication, they do support the results of our pulse-chase experiments which favour the alternative possibility that hrDNA represents one of the terminal stages of replication. It remains to be determined whether hrDNA is an obligate component of plasmid replication.

Earlier investigations[2,3] have shown that replication is initiated at a specific sequence on the supercoiled (form I) DNA molecule(Fig. 5*a*), the replication origin, and proceeds either

Fig. 5 Schematic diagram of structural forms in the replication of circular DNA, indicating the proposed place of hrDNA in the cycle. Supercoiled CCC form I DNA (*a*) is progressively unwound during progeny strand synthesis (*b*, *c*) (ref. 9). Separation of the parental DNA strands at a time before completion of progeny strand synthesis (*d*, *e*) generates two daughter molecules with gaps (arrows) in the newly-synthesised DNA strands[21]. These gaps are subsequently filled and the strands are ligated (*f*), thereby generating covalently closed circles lacking superhelical turns, that is, hrDNA. Maturation of the progeny circular molecules presumably involves the progressive introduction of negative superhelical turns (*g*) until the winding number typical of form I DNA (*h*) is reached.



unidirectionally or bidirectionally away from this site, causing the parental strands progressively to unwind and separate (Fig. *5b–d*). When the parental strands have been unwound completely, the progeny molecules separate (Fig. 5*e*). Initially they contain a "nick" or "gap" in the newly synthesised strand[21]. These nicked or gapped form II progeny molecules subsequently become converted to a covalently closed supercoiled form I species (Fig. 5*h*). Presumably, supercoils could be introduced into progeny molecules either before sealing of the closed circular form, or subsequent to an initial closing of the molecule.

Our finding that a closed circular monomeric DNA form lacking supercoils is an intermediate in plasmid replication suggests that newly separated progeny DNA molecules may be sealed to the closed circular form before the introduction of superhelical turns (Fig. 5*f*). Maturation of progeny DNA presumably involves the supercoiling of this species, possibly by means of specific DNA binding proteins and a swivel enzyme[22,23]. If this supercoiling step occurs by a series of nicking–closing reactions (that is, is quantal), molecules with superhelix densities intermediate between that of form I DNA and DNA lacking supercoils (that is, with properties similar to those of the molecules present in the lighter component of hrDNA) may be generated (Fig. 5*g*).

The occurrence of covalently closed circular DNA molecules lacking supercoils as intermediates in the replication of circular DNA seems to be widespread. hrDNA has been observed in various *E. coli* plasmids (K. Timmis and F. Cabello, unpublished experiments, Y. Kupersztock, personal communication, and Crosa *et al.*[25]) including ColE1, mini F (pSC138) (ref. 24) mini R6-5 (pSC135) (ref. 24), pSC134, F, and RSF1040. It has also been observed during the replication of mitochondrial DNA[26,27]. Also, completely replicated CCC progeny molecules of SV40 and polyoma DNA of similar appearance and lacking superhelical turns accumulate in cells treated with protein synthesis inhibitors[28–31], suggesting that such molecules may also be intermediates in the replication of these viruses.

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Covalently closed circular DNA molecules deficient in superhelical density as intermediates in plasmid life cycle

WE have described[1] the replication of the conjugative R plasmid, RSF1040, a deletion mutant of R6K (ref. 2). RSF1040 possesses two distinct origins and replication generally proceeds from one or the other in an asymmetrically bidirectional mode to a unique terminus[1], although in several instances molecules with two simultaneous initiations were observed[2]. During the course of examining the kinetic behaviour of replicating RSF1040 DNA (ref. 1), we observed ^{3}H-thymidine pulse-labelled material that had a greater buoyant density in CsCl–ethidium bromide gradients than did mature covalently closed circular (form I) plasmid DNA. The nature of this material ('heavy DNA') and its relationship to the replication cycle are the subjects of this report.

Our previous study[1] has shown that only a small fraction of the DNA labelled during a short pulse ($\leqslant$35 s) of ^{3}H-thymidine banded in the region with higher buoyant density than form I DNA. We were unable to find any 'heavy DNA' in 5–10-s pulses, whereas longer pulses (>35 s), always showed an appreciable amount of this material. Figure 1 shows the kinetics of DNA pulse-labelled for 50 s and its subsequent fate after a chase of unlabelled thymine plus thymidine as revealed by CsCl–ethidium bromide centrifugation of cleared lysates. Most of the DNA labelled during the 50-s pulse banded at a position intermediate between the ^{14}C-prelabelled forms I and II (open circular) DNA (Fig. 1*a*). Approximately 9% of the pulse-labelled material, however, banded heterogeneously in two peaks at buoyant densities significantly greater than that of form I DNA. After a 60-s chase (Fig. 1*b*) the fraction of DNA occupying an intermediate density between forms I and II moved to lower density regions (that is, had replicated to a greater extent[1]). Concomitantly the fraction of DNA banding in the form I region had increased to about 15% of the total replicating DNA and now occupied a position closer to, but still perceptibly more dense than, form I DNA. Subsequently, after a 180-s chase (Fig. 1*c*) virtually all the pulse-labelled DNA had disappeared from intermediate densities and was found in the form II as well as the form I positions of the gradient. Note, however, that although the fraction of ^{3}H-thymidine-labelled DNA banding in the form I region had increased substantially, it still exhibited components slightly denser than mature non-replicating form I DNA.

Our observations that the 'heavy DNA' was not found when very short pulses were given prompted us to examine the fate of DNA labelled during a 25-s pulse and during the subsequent chase. Figure 2 concentrates solely on fractions adjacent to the form I region, including the regions of higher buoyant densities. These data indicate that some of the pulse-labelled material bands in the CsCl–ethidium bromide gradient at a buoyant density greater than form I DNA. As in the longer pulse of Fig. 1 this 'heavy' material, although heterogeneous, seems to contain two components banding at 7 and 3 fractions respectively, ahead of form I

DNA. After a 30-s chase, there is a net increase in material banding closer to the position of form I DNA, as well as an increase in the 'heavy DNA' sedimenting heterogeneously at about 7 fractions ahead of form I DNA. The subsequent chase samples showed a reduction in the 'heavy DNA' with concomitant increase in the regions closer to form I DNA. The data indicate that there is a dynamic shift in buoyant density of pulse-labelled DNA from that of the 'heavy DNA' to that which is indistinguishable from form I DNA. The observation that there is a transient appearance of the 'heavy DNA' during plasmid DNA replication and that the material is subsequently chased into form I DNA suggests that it represents a replicative intermediate form of the plasmid DNA.

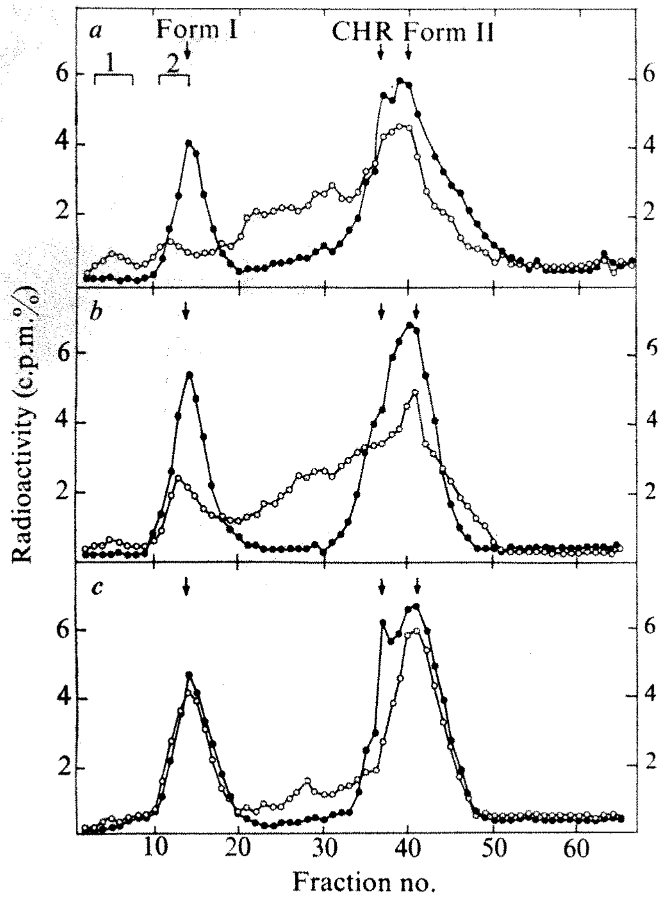
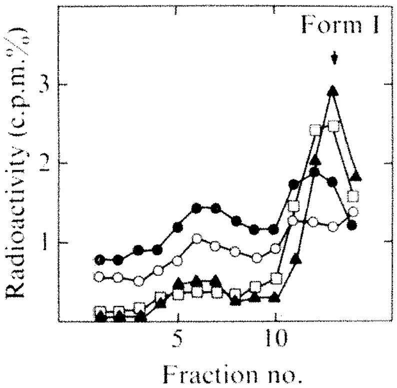


Fig. 1 CsCl–ethidium bromide density gradient analysis of a 'long' pulse-chase experiment. *Escherichia coli* K12 W1485-1 *F⁻thy⁻nal^r* containing RSF1040 (ampicillin resistant; *Ap^r*)[2] was used throughout this study. Cells were grown at 37° C with aeration in a 100-ml culture of M9 medium containing 0.5% glucose, 0.2% Casamino acids, 2 μg ml^{-1} thiamine, 3 μg ml^{-1} thymine and 0.6 μCi ml^{-1} 2-^{14}C-thymine, to a density of 2×10^8 cells per ml, collected by centrifugation at 25 °C, resuspended into an equal volume of prewarmed medium lacking thymine and incubated at 37 °C for 30 min (ref. 1). The culture was cooled to 25 °C and pulse labelled at this temperature for 50 s with ^{3}H-thymidine (20 μCi ml^{-1}; 0.05 μg ml^{-1}) and then chased for various periods of time (pulse 50 s, panel *a*; chases 60 and 180 s panels *b* and *c*, respectively), by addition of unlabelled thymidine (400 μg ml^{-1}) and thymine (20 μg ml^{-1}). Incorporation was stopped by addition of sodium azide (5×10^{-2} M, final concentration) and by freezing in a dry ice–ethanol bath. Cell suspensions were thawed and, after centrifugation, washed with cold TES buffer (50 mM Tris-HCl, 5 mM EDTA, 50 mM NaCl, *p*H 8) and resuspended to a density of 5×10^{10} cells per ml. The cells were lysed by the procedure of Clewell and Helinski[18] using 0.05% Triton X-100, and 'cleared lysates' were prepared; 5.2 ml of such 'cleared lysates' were mixed with 4.9 g CsCl and 2 mg ethidium bromide and centrifuged at 40,000 r.p.m. for 64 h at 15° C in a 50 rotor of a Beckman I 3-50 ultracentrifuge. Five-drop fractions were collected from the bottom of centrifuge tubes into microtitre trays and 10-μl samples of each fraction were spotted on to 3MM Whatman filter paper circles, which were successively treated with cold TCA and ethanol and dried to determine the ^{3}H (○) and ^{14}C radioactivity (●) present in every fraction by counting in a liquid scintillation counter. In all panels the density increases from right to left. Arrows indicate the positions of covalently closed circular mature (form I) DNA and open circular or nicked (form II) DNA. The chromosome (CHR) bands two or three fractions ahead of plasmid form II DNA due to GC ratio differences[2].

The appearance of DNA with a buoyant density greater than that of non-replicating covalently closed circular DNA could be due to the presence of RNA complexed to replicating DNA or result from a difference in superhelical density[3]. To distinguish between these two possibilities, two pools (1 and 2, see Fig. 1*a*) were purified from a 50-s pulse-labelled lysate by centrifugation through a CsCl–ethidium bromide gradient. The purified DNA from each pool was treated with RNase at a low salt concentration[4], and treated with pronase. Treated and untreated DNA were recentrifuged in CsCl gradients containing the dye propidium iodide in place of ethidium bromide, to increase the resolution of various DNA species[5]. Figure 3 shows that the banding of pools 1 (*a* and *b*) and 2 (*d* and *e*) DNA was unaffected by RNase–Pronase treatment. The small amount of form II DNA present was similar in both treated and untreated DNA samples, and presumably reflected nicking which occurred during experimental manipulation. Note, however, that centrifugation of both pools in neutral CsCl density gradients did show a very small, but reproducible, increase in buoyant density (0.001±0.0001 g cm^{-3}, average of 6 experiments) over that of ^{14}C-labelled non-replicating form I DNA, which disappeared after RNase–Pronase treatment (our unpublished results). These results indicate the presence of RNA in 'heavy' replicating DNA. This RNA is not covalently linked to the closed circular DNA component since the RNase treatment does not produce nicking of this material. Whether this RNA is of functional significance remains to be shown, but certainly its presence was insufficient to account for the banding behaviour of pools 1 and 2 DNA in CsCl gradients containing intercalating dyes. We concluded, therefore, that the differences in band position arose from differences in superhelical density between pool 1, pool 2 and non-replicating form I DNA.

Fig. 2 CsCl–ethidium bromide density gradient analysis of a 'short' pulse–chase experiment. Conditions were as described in Fig. 1, except that cells were pulse labelled with ^{3}H-thymidine for 25 s and subsequently chased with unlabelled thymidine plus thymine; samples were withdrawn after various periods of time. The figure concentrates solely on fractions adjacent to the form I region and those of the region of higher buoyant densities. ○, 25-s pulse; ●, 30-s chase; □, 60-s chase; ▲, 180-s chase.



Treatment of pools 1 and 2 with the untwisting enzyme of rat cells (B. McConaughy and J. Champoux, unpublished) which untwists superhelical DNA to a covalently closed circular DNA species with no superhelix turns (relaxed or zero turns DNA), confirmed the view that these pools consisted primarily of molecular species containing a

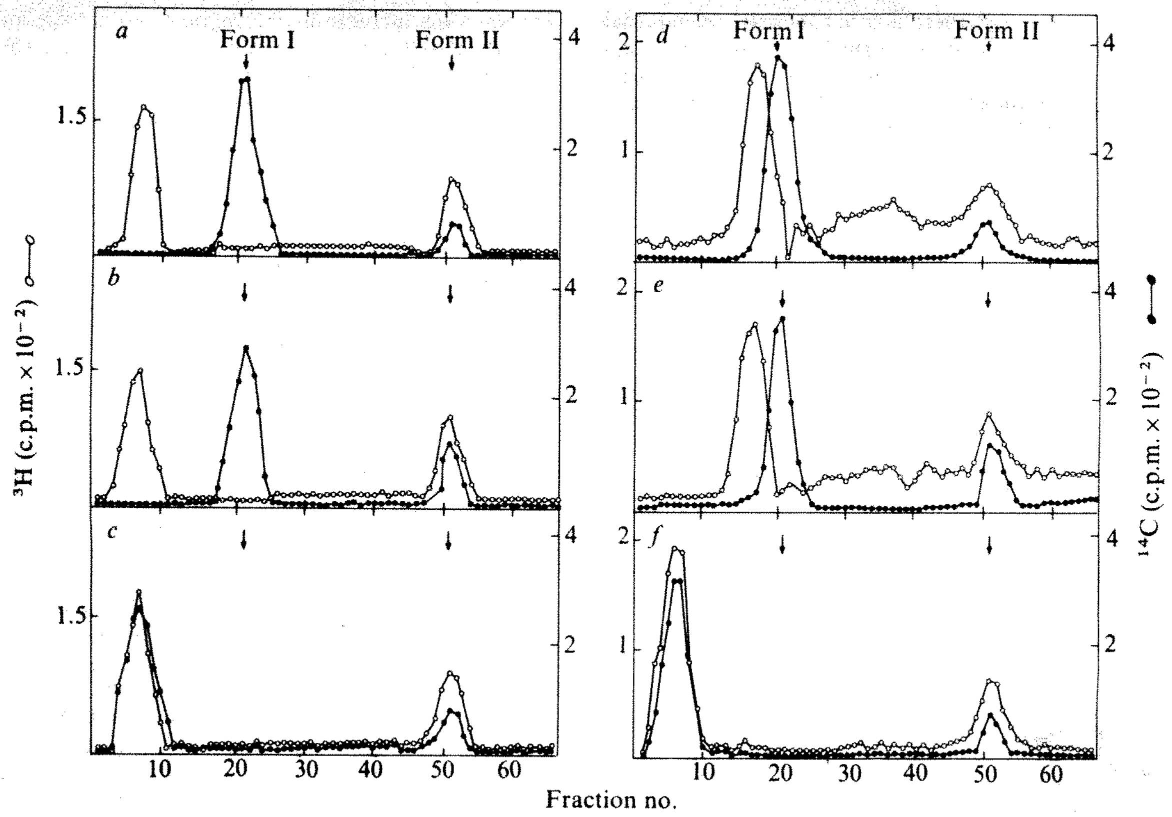


Fig. 3 RNase A and untwisting enzyme treatment of purified 'heavy' replicating RSF1040 plasmid DNA. A 400-ml culture of *E. coli* K12 W1485-1 (RSF1040) was pulse labelled with ^{3}H-thymidine (20 μCi ml^{-1}; 0.05 μg ml^{-1}) for 50 s. All subsequent manipulations were as described in Fig. 1. Centrifugation of the cleared lysate obtained in a CsCl–ethidium bromide density gradient gave a profile essentially similar to that of Fig. 1*a*. Pools 1 and 2 of 'heavy' replicating DNA were extracted separately with isopropanol (six times) to remove the ethidium bromide, and dialysed against 16 mM Tris-HCl, 5 mM EDTA, 16 mM NaCl, *p*H 8. RNase treatment consisted of the incubation of 50 μl pool 1 (or pool 2) DNA mixed with 0.1–0.5 μg ^{14}C-labelled form I RSF1040 DNA plus 1 mg ml^{-1} RNase A (Worthington) at 37 °C for 60 min (ref. 14). The RNase was subsequently removed by incubation with self-digested (45 min at 37 °C) Pronase (1.25 mg ml^{-1}). Untwisting enzyme treatment was carried out by incubation of 50 μl pool 1 (or pool 2) mixed with 0.1–0.5 μg ^{14}C-labelled form I RSF1040 DNA and sufficient enzyme to completely untwist 15.0 μg DNA in 30 min at 37 °C in the standard assay conditions (B. McConaughy and J. Champoux, unpublished). After incubation the reaction was terminated by dilution with 0.01 M Tris-HCl and the addition of CsCl. Untreated controls consisting of the reaction mixtures without the enzyme treatments were also incubated for each of the reaction mixtures. All samples were then analysed by centrifugation in a CsCl–propidium iodide density gradient (4.8 g CsCl, 2 mg propidium iodide (Calbiochem) and 5 ml TES buffer). Centrifugation was at 40,000 r.p.m. at 15 °C for 64 h in a 50 rotor. Fractions were collected directly on to 3MM Whatman filter circles and treated and counted as described in Fig. 1. ^{3}H-labelled pool 1 plus ^{14}C-labelled form I DNA: *a*, untreated samples; *b*, RNase–Pronase treatment; *c*, untwisting enzyme treatment. ^{3}H-labelled pool 2 plus ^{14}C-labelled form I DNA: *d*, untreated sample; *e*, RNase–Pronase treatment; *f*, untwisting enzyme treatment.

reduced superhelix density. A sample of pool 1 together with added ^{14}C-labelled form I DNA was treated with untwisting enzyme and banded in a CsCl–propidium iodide density gradient (Fig. 3*c*). The ^{14}C-labelled form I DNA which, after the untwisting enzyme treatment was converted to a form with zero superhelix turns, now banded at a buoyant density identical to that of pool 1 DNA which was unaffected by the treatment (compare Fig. 3*c* with the untreated control in Fig. 3*a*). The conclusion is that 'heavy' pulse-labelled DNA from pool 1 consists of covalently closed circular DNA with no (or few) superhelix turns. Indeed, examination of pool 1 in the electron microscope showed only open ring forms, and this material sedimented slightly ahead of form II DNA in neutral sucrose gradients (data not shown) as expected for a covalently closed circular DNA of those topological characteristics. Treatment of material from pool 2 with untwisting enzyme, as expected, converted it to a molecular species with a density identical to that of pool 1 DNA or untwisting enzyme-treated form I DNA (Fig. 3*f*). Since the distance between the open and closed forms of pool 2 DNA was about 10% greater than the distance between forms I and II non-replicating plasmid DNA (Fig. 3d) one can calculate (refs 6 and 7, and our unpublished results) that the molecules of newly synthesised plasmid DNA banding at the position of the fractions of pool 2, on average, have a deficiency in the superhelical density of about 20%, compared with non-replicating form I DNA. It is clear from Fig. 2, however, that covalently closed circular RSF1040 plasmid DNA molecules encompassing a whole range of superhelical densities (from zero to that of form I DNA) may be isolated depending on the time of sampling after the ^{3}H-thymidine pulse.

Pulse-labelled DNA banding at positions more dense than mature form I DNA has been found in all plasmid systems examined by us so far (in addition to RSF1040, we have determined its presence in replicating DNA of plasmids RSF1010, RSF1030, R6K and ColE1; our unpublished results) and has also been characterised in pulse-labelled pSC101 (ref. 8).

Our results with RSF1040 (ref. 1), and those reported for other covalently closed circular DNA systems as ColE1 (refs 9 and 10, and L. Katz, P. H. Williams, S. Sato, R. W. Leavitt and D. R. Helinski, unpublished), pSC134 (ref. 11), SV40 (refs 12 and 13), polyoma[14] and mitochondrial DNA (ref. 15) indicated that DNA synthesis occurred on a covalently closed circular template. The parental strands of replicating molecules were continuously unwinding during the replication cycle, presumably by a nicking and closing mechanism involving endonucleolytic cleavage or a DNA untwisting enzyme[16,17]. It is assumed that the unwinding of parental strands proceeds until all of the topological turns are removed and the daughter molecules can then separate, with at least one of the progeny molecules containing a nick and possibly a gap. A consequence of this assumption is that the nicked (or gapped) progeny (form II DNA molecules) will be closed subsequently and converted to form I DNA molecules, the end product of replication. Our earlier data[1], as well as those of other groups[9,12,14], indicated that form II DNA was indeed an intermediate during the replication of a covalently closed DNA molecule. The data presented in this paper suggest an intermediate stage between form II and form I DNA as one of the terminal events of replication. Form II DNA is presumably sealed to a covalently closed species with no superhelical turns. These transient forms appearing as 'heavy' DNA are in turn modified by the progressive introduction of negative superhelical turns until the superhelical density of form I DNA is achieved. This maturation process is presumably carried out through a series of nicking and sealing events, or by a protein or protein system which can introduce superhelix turns. Whether this type of transition is an obligatory step for all replicating form II DNA molecules remains to be demonstrated.

Earlier studies have shown that intracellular SV40 viral and PM2 phage DNA have lower superhelix densities than corresponding mature viral DNA (refs 7 and 19). Moreover, replicating SV40 and polyoma DNA accumulate in cells treated with protein synthesis inhibitors as closed circular molecules with very few turns[20–23]. Covalently closed molecules with low superhelical density have also been described for replicating mitochondrial DNA (refs 24 and 25).

The mechanism whereby superhelix turns may be introduced as a terminal step into newly replicated DNA is not established, although the formation of closed circular DNA of low superhelical density seems to be a common event during the life cycle of plasmid and viral DNA and may also prove of significance in the replication of circular molecules in other biological systems.

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Role for small nuclear RNAs in "programming" chromosomal information?

ALTHOUGH small nuclear RNAs (snRNAs) have been studied extensively[1,2], almost nothing is known about how they function in cells. I present here the results of a detailed study of the behaviour of snRNAs during mitosis, which has uncovered what may be the first important clue as to their function. I suggest, from observations of the pattern of snRNA association with mitotic chromosomes, that snRNAs are involved in specifying which genes are transcribed. Between 4 and 10 electrophoretically distinct snRNAs are found in species from protozoa to mammals, ranging from 65 to 200 nucleotides long. The snRNAs, which generally comprise less than 0.5% of total cellular RNA, are largely confined to the nuclei during interphase; some, however, are probably present in the interphase cytoplasm—although at concentrations much below that in the nucleus[3]. Such molecular characteristics as base compositions, methylation patterns and nucleotide sequences have been determined for at least some snRNAs, but have provided no recognisable clues regarding function. No evidence is available that snRNAs are part of the cellular translation machinery, as is true for the more abundant transfer, ribosomal and messenger RNAs, and their metabolic stability argues against a function as primers in DNA replication[4]. It has been suggested that certain snRNAs are related to ribosomal RNA processing[2], cell proliferation[5] and gene expression—but no data in support of these views are definitive. The snRNAs have been shown to be released to the cytoplasm during division, then to return virtually intact to the post-division daughter nuclei[6,7].

By transplanting ^{3}H-uridine nuclei between directly labelled and unlabelled amoebae (*Amoeba proteus*) two or more times, it is possible to rid the nucleus (and the final recipient cell) of virtually all radioactive RNAs except the snRNAs (ref. 3). Because they are thus the only radioactive molecules in the cell, this technique allows us to follow clearly the behaviour of snRNAs during all subsequent experimental manipulations.

When an interphase cell containing a nucleus serially transplanted in this way is examined autoradiographically, the radioactivity is found to be almost entirely (>95%) in the nucleus (Fig. 1). The radioactivity seems to be uniformly distributed through the nucleus but, in fact, careful electron microscopic examination[8] shows that the concentration of ^{3}H is slightly higher over chromatin than over nucleoplasm but that the bulk of radioactivity is, nevertheless, non-chromosomal.

When a cell like that in Fig. 1 enters mitosis, much of the label is lost from the nucleus as the nuclear envelope breaks down, the nucleoli disappear, and the chromosomes condense. At metaphase the radioactivity is uniformly distributed through the cytoplasm, and virtually no label is associated with the chromosomes (Fig. 2). Thus, regardless of what may be the case at earlier mitotic stages, when the chromosomes are maximally condensed, essentially no snRNAs are associated with them.

The most unexpected result is observed just a few minutes later. At about the middle of anaphase the snRNAs are

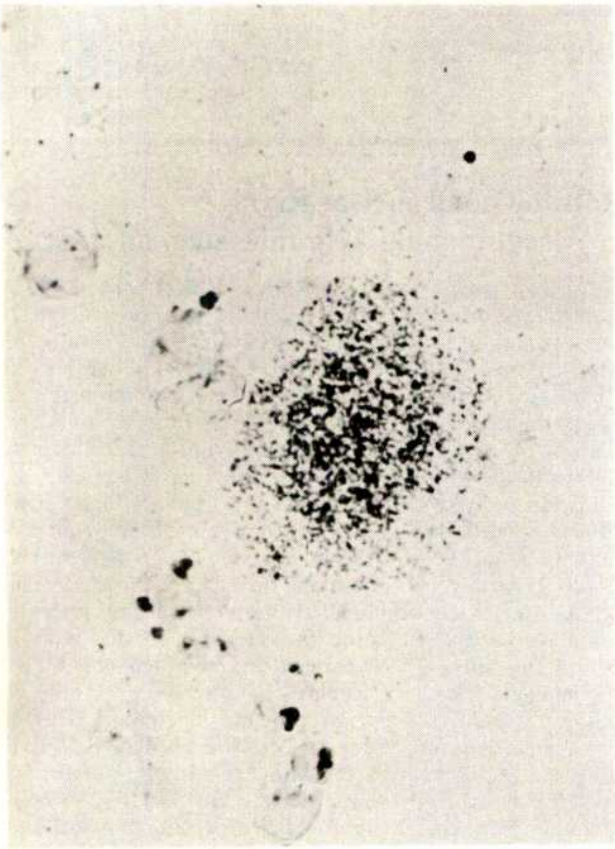

Fig. 1 Portion of an interphase cell (~ ×700 magnification) and its autoradiogram after the cell has been fixed and squashed with a coverslip containing a hanging drop of 45% acetic acid. The cell contains a nucleus transplanted[15] through two unlabelled cytoplasms at 2-d intervals after the original donor cell had been labelled with ^{3}H-uridine for 2 d and then fed unlabelled food for 3 d. Autoradiography was performed with Kodak's NTB-3. Note the virtual absence of label over the cytoplasm. The lesser amount of label around the nuclear periphery is attributable to the presence of a ring of nucleoli, which are more lightly labelled than the rest of the nucleus in these conditions.

found to be associated with the chromosomes to an enormous extent (Fig. 3), far more than had been the case during interphase. Thus, as the chromosomes begin to decondense, large numbers of sites apparently become available for the binding of snRNAs. As mitosis continues and the nuclear envelope reforms, the snRNAs begin to dissociate from the chromosomes but remain within the newly-formed nuclei. Some time during the first few hours of interphase, the distribution of snRNAs again becomes like that of the premitotic cell (Fig. 1); that is, although almost entirely nuclear, only a small proportion of snRNA is associated with chromatin.

In preliminary electron microscopic studies (L. G., G. E. Wise, and C. Ko, unpublished), not only have these results been more clearly observed, but late prophase chromosomes have also been found to have a large amount of snRNAs associated with them. Thus, incompletely condensed (or decondensed) chromosomes apparently have a particular affinity for snRNAs.

From what is known of the behaviour of acidic nuclear proteins (ANPs) during mitosis and the role of 'quantal' mitoses in differentiation[9,10] I propose a simple model of mitosis-associated rearrangements of chromosomal macromolecules, and the significance of those rearrangements—borrowing from the ideas of Holtzer[9] and Gurdon[10]. In amoebae, at least, the nuclear RNAs return from the cytoplasm in their entirety to the post-division nuclei within 15 min or less of the completion of telophase[11], whereas all of the ANPs (which are also released to the cytoplasm during the early stages of mitosis) take about 3 h (ref. 12). Thus, at least superficially, the snRNAs seem to be displaced from their chromosomal associations by the returning ANPs. Because we expect that only a small proportion of a cell's genes are being transcribed at any one time, the fact that only a small proportion of snRNAs remain associated with interphase chromatin suggests that snRNAs on chromosomes have a positive role in gene expression—presumably serving (perhaps by some specific base-pairing mechanism) to open up selected loci to transcriptive activity. (Such a model suggests that the rate of transcription ought to be unusually high immediately after mitosis, and examination of some data in connection with other experiments (L. Goldstein and C. Ko, unpublished) indicates that that may well be the case.) There is evidence that some small nuclear RNAs may stimulate transcription in specific ways[13].

According to this model, specificity in locus selection need not reside in the snRNAs, but could well be a function of the ANPs, as has been frequently suggested (see, for example, ref. 14); the ANPs could act by displacing snRNAs from the selected chromosomal loci. That ANPs act thus in the 'programming' of chromosomes in the course of cell differentiation has been proposed by Holtzer[9] and Gurdon[10], who have noted that in spite of otherwise

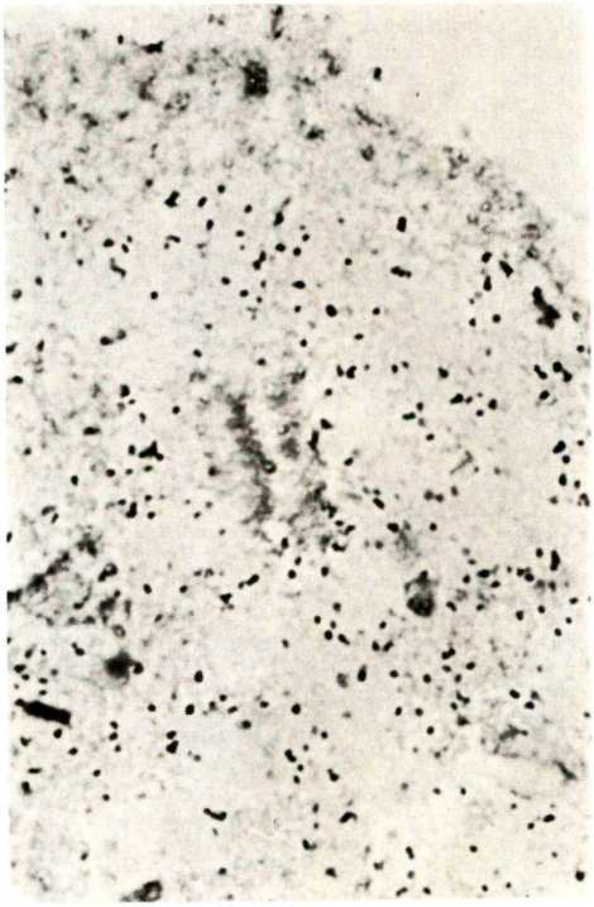

Fig. 2 Cell (~ ×1,500), from the same population as cell in Fig. 1 but in mitosis at time of fixation, at the very beginning of anaphase (separating chromosome sets in middle of the picture). Cell sectioned at about 8 μm and stained with Giemsa. Note virtual absence of autoradiographic grains over chromosomes.

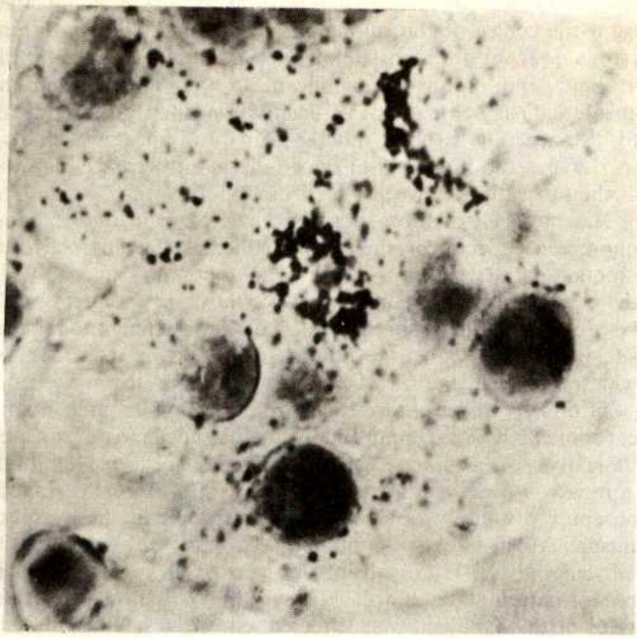

Fig. 3 As Fig. 2, except cell (~ ×2,000) is in mid-anaphase. (Round, dark-staining bodies are food vacuoles.) The stained chromosome sets are obscured by the high concentration of autoradiographic grains.

favourable conditions certain kinds of cells do not change their phenotype unless they undergo mitosis (called a 'quantal' mitosis) subsequent to the occurrence of the differentiation-inducing condition. Such an hypothesis seems to require that the primary event leading to differentiation is, for example, an environmentally-induced change in the cytoplasmic spectrum of certain latent ANPs that cannot immediately associate with chromosomes to affect transcriptive activity either because the nuclear envelope is a barrier or because ANPs already associated with the chromosomes cannot be displaced. When the affected cells enter into mitosis, however, the 'old' ANPs (and snRNAs) are discarded from the chromosomes, thus ostensibly enabling others from the cytoplasm to associate with the decondensing chromosomes from anaphase onward.

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Kinetics of triplet–triplet energy transfer and intramolecular distances in enzyme–inhibitor complexes

WE wish to report an application of the relationship between the kinetics of triplet energy transfer and donor–acceptor geometries to biomolecular systems. Our study illustrates the usefulness of measurements of this type in estimating molecular distances and providing a measure of variability within enzyme–inhibitor complexes.

The sensitivity of radiationless transfer of triplet electronic excitation to distance[1] prompted its use for exploring the proximity of chromophores in nucleic acid[2] and protein complexes[3], and triplet–triplet energy transfer has been demonstrated in various biomolecular systems in rigid solution[4–7]. In favourable cases the transfer has been quantitated from the quenching of the donor phosphorescence in terms of a transfer efficiency. Although it has been possible to establish an upper limit on the distance between donor and acceptor groups from "active sphere" radii[1] for transfer in model systems, the lack of an experimentally determined distance dependence for the efficiency has limited the usefulness of triplet transfer as a measure of the separation between chromophores. Furthermore, although the transfer seems efficient when it proceeds at a rate which is more rapid than the normal triplet decay rate (0.1–10^2 s^{-1}), studies with solutions[8,9] and molecular crystals[10] indicate that triplet transfer occurs at a rate orders of magnitude more rapid than this as molecules approach van der Waals' contact. The more rapid transfers corresponding to shorter intermolecular separations can be monitored if the transfer kinetics, rather than transfer efficiencies, are measured directly.

Rates of triplet–triplet transfer have been determined from the decay of the phosphorescence of the donor following laser-pulsed excitation[11,12]. An exponential dependence of the transfer rate constant on distance of the form predicted by Dexter[13] has been found and empirical parameters relating transfer with distance have been established for several donor–acceptor pairs[11,12,14]. In our study the rates of energy transfer from a sulphonamide inhibitor to tryptophan residues in bovine and human B and C forms of carbonic anhydrase have been measured and translated into the corresponding distances.

When the energy donor (*m*-acetylbenzene sulphonamide)[8] bound at the active site of all three carbonic anhydrases is excited selectively at 330 nm, the steady state phosphorescence of the sulphonamide is replaced by a sensitised tryptophan phosphorescence. The marked reduction, or quenching, of the donor emission indicates that the efficient transfer which had been demonstrated earlier for the bovine enzyme[3] is also operative in both human forms of the enzyme. Efficient triplet transfer with the same inhibitor has also been observed (A. Maki, personal communication) during an optical detection of magnetic resonance (ODMR) study of these systems.

Although the steady state phosphorescence results establish that transfer is efficient in all three enzymes, our flash experiments reveal that the transfer rates differ significantly. The decay of the phosphorescence of the donor after an exciting pulse at 337 nm from a small N_2 laser is depicted in Fig. 1. Transfer occurs with a rate constant of 2.4×10^4 s^{-1} in the bovine enzyme; 10^4 s^{-1} for 70–80% of the transfer in the human C enzyme with the remainder occurring more rapidly; and at a rate which was too rapid for reliable detection ($> 10^5$ s^{-1}) in our experiments with the human B form. Although rates of 10^4–10^5 s^{-1} are sufficiently large compared with the normal donor decay (half value $= 3.3 \times 10^2$ s^{-1}) to produce the efficient transfer observed, they are orders of magnitude less than would be anticipated for molecules in van der Waals contact.

Ignoring the potential contribution of the relative orientation

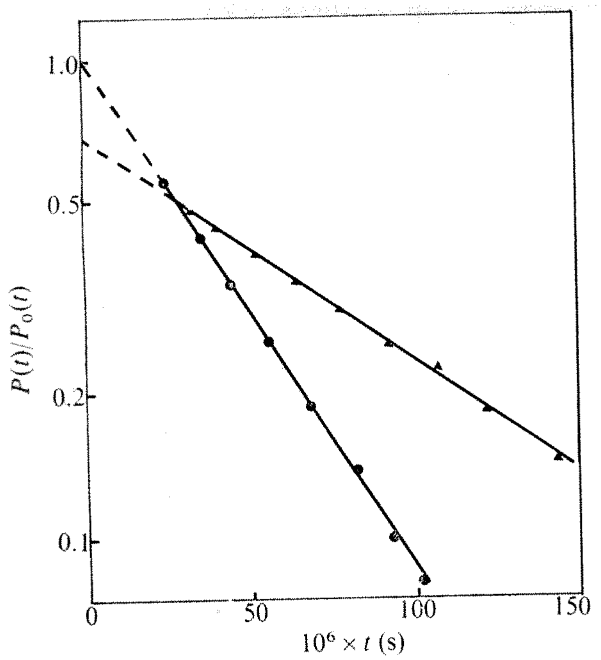

Fig. 1 Decay of the phosphorescence of *m*-acetylbenzene sulphonamide (donor) due to energy transfer to tryptophan residues in human carbonic anhydrase C (▲) and bovine carbonic anhydrase (Sigma) (●). The sulphonamide inhibitor was synthesised from *m*-acetylbenzene sulphonylfluoride (Aldrich) by addition of ammonia[3]. Kinetic measurements were made with solutions of native enzyme (1.2×10^{-3} M) plus inhibitor (1×10^{-3} M) in 1:1 ethylene glycol: phosphate buffer (0.1 M, *p*H 8.0) at 77 K. The sulphonamide inhibitor was excited selectively at 337 nm with a pulse (< 20 ns) from a molecular nitrogen laser[16]. The decay of the donor phosphorescence after pulsed excitation was monitored at 390 nm. The donor phosphorescence intensity in the absence, $P_0(t)$, and presence, $P(t)$, of energy transfer can be represented as

$$P_0(t) = P(0)\exp(-t/\tau_0)$$
$$\text{and } P(t) = P(0)\exp(-t/\tau_0)\sum_i \alpha_i \exp(-k_{Ti}t)$$

where τ_0 is the donor lifetime in the absence of transfer and α_i is the fraction of donor molecules which transfer with a rate constant k_{Ti}. The contribution to the decay from energy transfer was evaluated from

$$\ln P(t)/P_0(t) = \ln\{\sum_i \alpha_i \exp(-k_{Ti}t)\}$$

which for a single rate constant reduces to

$$\ln P(t)/P_0(t) = -k_T t$$

of the donor and acceptor to the transfer, we have translated the measured triplet–triplet energy transfer rate constants k_T into distances using the relationship

$$k_T = 8.3 \times 10^{15}\exp(-2.63r)$$

found for transfer between acetophenone and indole in solid solution[14]. This results in a distance or an average distance of 10.1 Å from the centre of the sulphonamide to that of one or more tryptophan residues in the bovine enzyme, 10.4 Å for the slowly transferring component in the human C enzyme and < 9.5 Å in the B form. The absolute distances must be considered as somewhat uncertain because the magnitude of the role of molecular orientations in the transfer process is not known. Using the coordinates (K. K. Kannan, personal communication) for the structure of the crystalline human C enzyme[15], and with the assumption that our energy donor binds in a manner analogous to that of a very similar sulphonamide[15], we have estimated the distances from the inhibitor to the tryptophan residues in the enzyme. The two closest tryptophan residues lie at a distance (10.3 and 10.6 Å) from the centre of the sulphonamide which is similar to that estimated from our transfer rate measurements for the enzyme in rigid solution. More significant in this respect than the absolute measurements is the sensitivity of the transfer kinetics to distance. The twofold difference in rate constant found in the bovine and human C enzyme reflects a difference of 0.3 Å in the separation between the chromophores.

The presence of more than one transfer rate in the phosphorescence decay of the donor indicates the absence of a unique spatial relationship between donor and acceptor. In the enzyme–inhibitor complexes a variation in geometry could be produced either from the presence of more than one binding site within the "substrate binding cavity"[15], or from a more widespread distribution of conformations for the proteins in solution. It is significant that in the sulphonamide–bovine carbonic anhydrase complex a single transfer rate constant indicative of a unique geometry for the enzyme–inhibitor complex is observed. A variation of > 0.2 Å in the donor–acceptor distance would have been detected in these experiments. Most inhibitor-containing enzyme molecules in the ethylene glycol–H_2O solvent must exist in a well defined conformation state. It will be interesting to compare transfer rates of this type with measurements in the crystalline state to ascertain whether the transfer rate constants reflect very similar geometries.

We feel that the sensitivity of kinetics of triplet energy transfer in exploring the variability within biomolecular structures and in detecting subtle changes in distance recommends the use of measurements of this type in the investigation of biomolecular conformations.

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Stereoselectivity of β irradiation of D,L-tryptophan in aqueous solution

A POSSIBLE connection between the molecular asymmetry of living matter and the intrinsic asymmetry of elementary particles[1] was first suggested by Vester[2]. All the experiments based essentially on the non-conservation of parity in weak interactions[3,4] can be classified as follows: (1) It is assumed that

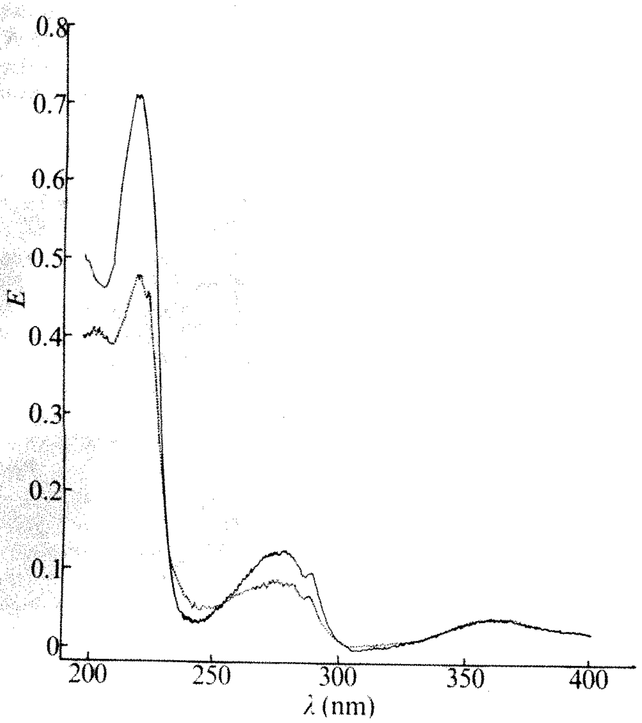

Fig. 1 UV-absorption spectrum for irradiated (. . . . . .) and non-irradiated (———) tryptophan samples ($c = 10^{-5}$ mol l^{-1}).

L- and D-enantiomers are not exact mirror images of each other but differ in their scalar physicochemical properties[5,6]. Parity-violating forces originated by weak interactions could in principle give rise to different bond strengths in enantiomer molecules. If the recently discovered neutral weak currents turn out to be of vector *and* axialvector type—an opinion favoured by present neutrino experiments[7–9]—there is necessarily an interference between parity-conserving electromagnetic interaction and parity-violating weak interaction; it might, however, be very small[23]. The question whether a particular molecular quantum state does allow for the actual occurrence of a seizable parity-violating effect has, to our knowledge, not yet been settled. (2) It is supposed that the almost exclusive existence of one enantiomer in the biosphere is due to the asymmetric interaction of longitudinally polarised β rays with molecules (interaction either with racemic mixtures or with molecules undergoing reaction to form asymmetric compounds) during the early history of the earth. With this view it is believed that an induced optical activity is a result of the natural radiation from isotopes in the earth's crust[4,10,11]. Experiments which aimed to generate optical activity in this manner are summarised in Table 1.

In an attempt to assess the validity of the latter hypothesis we investigated the irradiation of D,L-tryptophan with β rays from ^{32}P (mean energy of β rays: 0.57 MeV, degree of polarisation: 93.9%) in homogeneous solution (5 mg D,L-tryptophan in 100 ml twice-distilled water). Sixteen aliquots (2 ml) of this solution were filled into Pyrex ampoules. 5 mCi ^{32}P (as phosphate in 0.02 N HCl carrier-free in 0.2 ml) were added to eight of these ampoules; the remaining eight (controls) were diluted with 0.2 ml 0.02 N HCl. All sixteen ampoules were sealed and left at −25 °C for 12 weeks. The energy absorbed during this period by the whole system would be 1.9×10^{20} eV. After thawing and opening the ampoules aliquots of 2 ml were withdrawn and diluted with distilled water to 20 ml. Samples were transferred directly to a quartz cell to measure the ultraviolet absorption spectrum at 200–400 nm and to determine the optical activity at 220 nm.

The procedure for determining angles of rotation in the millidegree range has been described in detail elsewhere[12]. Figure 1 shows that the overall decomposition of tryptophan by irradiation with ^{32}P after 12 weeks is about 33%. Figure 2 shows that the 'specific angle of rotation' at 220 nm is equal to -3.6 millidegrees cm$^{-1} \times 10^{-5}$ mol l^{-1} ($[\alpha] = -17{,}600$). Table 2 shows that the eight irradiated samples have a positive angle of rotation of $+0.7 \pm 0.4$ millidegrees (mean value of sixteen independent runs).

Table 1

Reference(s)	Investigated system	Irradiation source	Intensity	Time of irradiation	Analytical method	Result
15	Organic syntheses and decompositions involving free radicals and carbonium ions	β$^-$ emitters: ^{32}P ^{90}Sr/^{90}Y ^{52}Eu ^{108}Ag ^{110}Ag	30 mCi–1 Ci	5–2,900 min	α at Na–D line	No effect
17	Optically active and racemic substrates in solid state	β$^-$ emitter ^{104}Rh and electrons from accelerator	20–200 Ci		α at Na–D line	No effect
16	L-and D-tyrosine in aqueous solution	β$^-$ emitter ^{90}Sr/^{90}Y	0.36 mCi	1.5 yr	Ultraviolet absorption spectrum	D-tyrosine more decomposed
18	^{14}C-labelled racemic norvaline, alanine, dopa, aspartic acid, methionine in solid state	β$^-$ emitter ^{14}C, autoradiolysis		12–24 yr	ORD	No effect
19	Optically active and racemic norleucine, glycine, proline in solid and dissolved state	Bremsstrahlung from β$^-$ emitter ^{90}Sr/^{90}Y	61.7 kCi	1.34 yr	ORD between 250–630 nm and GC	No effect
20	L,D-phenylalanine, L,D-tyrosine, L, D-tryptophan	β$^+$ emitter ^{22}Na	0.8 μCi		Triplet positronium and annihilation time	D-isomers favour triplet state
21	D- and L-leucine	Left- and right-polarised electrons from accelerator			ORD, GC	Left-handed e$^-$ destroy D-leucine preferentially and vice versa
22	D,L-alanine, D,L-octanol solid and dissolved	μ$^+$ particles from accelerator			Formation of muonium and subsequent chemical reaction	No effect

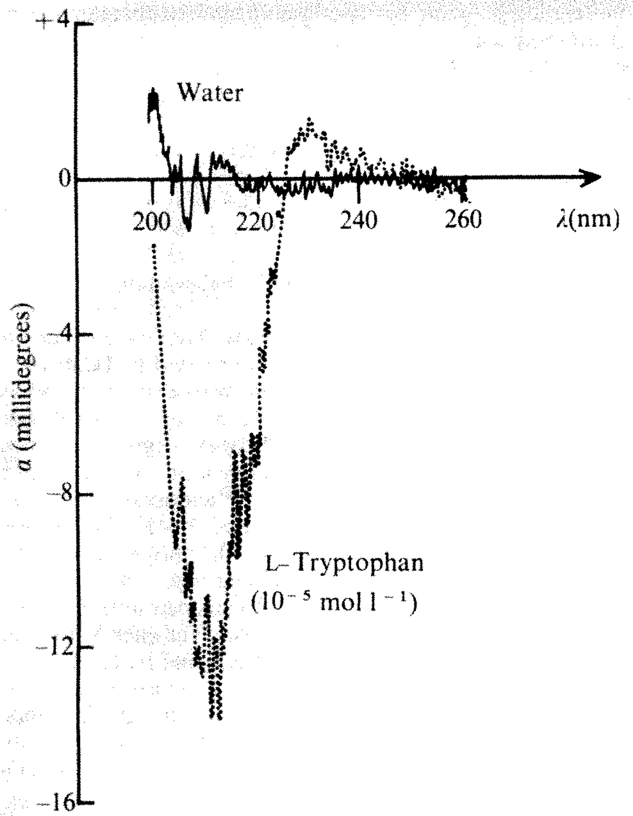

Fig. 2 Typical ORD of a diluted untreated sample of L-tryptophan in 0.002 N HCl ($c = 10^{-5}$ mol l^{-1}).

From the comparison of these values it seems that a preferential destruction of L-tryptophan has taken place, resulting in a relative enrichment of about 19% of the D-enantiomer. This is in qualitative agreement with previously reported experiments on the interaction of D- and L-tyrosine with β particles[16]. In our case, however, the L-enantiomer was decomposed to a greater extent than the D-enantiomer, whereas Garay[16] found that D-tyrosine was preferentially destroyed. (Note that the CD of tryptophan and tyrosine in ultraviolet are of opposite sign.) It may be that the different structure of these amino acids is of importance in connection with these results. Our experiments were designed to meet the criticisms of Garay's early work in which enantiomers had been studied in separate batches (which left uncertain the identity of the experimental conditions in the two cases); and the direct approach of irradiating a racemic mixture to produce optical activity had not been carried out.

Table 2

Date	Sample number	α at 22 °C and 220 nm (millidegrees)*
March 3, 1975	1	+0.8
	2	+0.4
	3	+0.2
	4	−0.2
March 4, 1975	1	+1.0
	2	+0.6
	3	+1.6
	4	+1.0
March 5, 1975	5	+0.8
	6	+1.0
	7	+1.0
	8	+0.4
March 6, 1975	1	+0.4
	2	+0.6
	3	+0.4
	4	+1.0

Mean α = +0.7±0.4 millidegrees.

*Data are already corrected and calculated from the statistically weighted recorded[12] α for each sample and reference (zero) pure solvent.

Recently, two additional results have been obtained concerning the origin of chirality in nature, Kovacs and Garay[13] reported a preferential influence of polarised β particles on the crystallisation of racemic compounds, which is difficult to understand in the light of existing classical theories. Furthermore, a particularly startling experiment has been reported by Merwitz[14]: Irradiation of solid L-, D- and D,L-phenylalanine with non-polarised γ particles had a discriminating effect on the decomposition of the target substance. Very large differences in the production of CO_2 from the main decarboxylation reaction were observed. This latter result cannot be interpreted on the basis of polarised particles as proposed in the original 'Ulbricht–Vester mechanism[4,10,11,15]. This result supports the above-mentioned view that there are differences in the scalar properties of enantiomers. As these energy differences are very small they may have been amplified in some kind of chain reaction.

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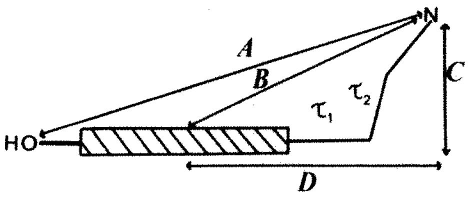
Corrigendum

In the article "Structural and conformational relationships between the enkephalins and the opiates" by A. S. Horn and J. R. Rodgers (*Nature*, **260**, 795; 1976) Fig. 1*c* is more correctly depicted as shown below and the mean value for the parameter *D* is 4.3 Å not 4.4 Å.



matters arising

Solar activity on August 12, 1975

ATTENTION has been called to the unusual number and proportion of Type III-RS (reverse slope) solar radio bursts observed on August 12, 1975 (ref. 1). We believe a simple explanation of these observations is suggested by comparison with $H\alpha$ filtergram movies obtained at Big Bear Solar Observatory on that date. We observed a number of the radio bursts to coincide in time with a homologous set of flares at the preceding edge of Mount Wilson spot group 19598. This group was at $\sim$ 45°W, thus our line of sight to the flares lay through the corona above the spot group. We suggest that the bursts were emitted as U bursts by an electron stream travelling from the flare site along a closed field line to the following part of the group. The emission at the plasma frequency on the preceding side of the field line could not propagate through the coronal density enhancement over the spot group to Earth. Only on the following side, where there was no intervening region of higher density, could the emitted radiation be observed. Therefore the bursts were seen only where the electrons were moving downward in the corona, producing reverse slope bursts. A detailed study of these events is in preparation.

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Oscillations in tropical ecosystems

BIGGER[1] has provided examples to show that population oscillations do in fact occur in the tropics. My purpose here is to show that his observations actually provide evidence in favour of the suggestion[2] that the linear connectivity of complex ecosystems is low, even though they contradict a deduction that was drawn from this.

There are essentially three types of oscillations which we might expect to observe in an ecosystem. Two of these are produced by interactions within the system; these are Lotka–Volterra oscillations around a stable steady state and limit cycle oscillations around an unstable steady state. The third possibility, which was not taken into account by Saunders and Bazin, is forced oscillations, in which a system which would otherwise be in a stable steady state oscillates either in response to a cyclic environmental perturbation or else on account of some autonomous oscillation of one species.

When oscillations are caused by interactions, the periods are chiefly determined by the parameters of the system. In such a case we would not expect the periods to be almost all very nearly one or two years; this is much more likely to indicate that the oscillations arise from seasonal variation in the environment. Bigger's observations are thus not in conflict with the suggestion that the structure of tropical ecosystems is not such as to produce oscillations. Moreover, that the response of the system to a periodic perturbation is a stable oscillation with the same period is evidence that the system is overdamped; were this not the case we would expect there to be oscillations with different periods (determined by the parameters of the system and not, in general, equal to one generation length) superimposed on the annual cycle.

The evidence, then, still supports the contention that the linear connectivity of complex ecosystems is low and that it is this that accounts for their stability. It follows that it is not likely to be profitable to think of complex ecosystems as generalised Lotka–Volterra systems which can be adequately analysed in terms of the linear parts of their interactions. Indeed, if it is true that even the lynx cycle in Canada is a forced oscillation[3], then the Lotka–Volterra equations themselves may be directly applicable almost nowhere in ecology, though they may remain useful for conceptual purposes.

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Evidence for polygenes

THOMPSON[1] has suggested that the generally held belief among quantitative geneticists that quantitative variation is determined by a large number of loci is an assumption which "unfortunately, some have come to think of . . . as an established fact". Fisher[2] considered in detail the hypothesis that some traits were determined by a large number of Mendelian factors. The assumptions made in that paper are the consequence of this hypothesis. He showed, as he says[3], "that the heredity behaviour to be observed does not become more complex" as the number of factors is increased. The evidence available, so far, favours this hypothesis.

Thompson[1], however, argues that "there is no compelling reason to think that the total number of loci affecting a quantitative character is exceptionally large". He believes that a "relatively small" number of genes could account for the genetic variance of a quantitative character; however, there are a number of published cases where a large number of loci is indicated.

Consider 3 loci, each with two equally frequent alleles, no dominance and equal effects. With environmental effects the distribution of the trait determined by these loci will be approximately normal. If 10% of the population are selected as parents, then after two generations the parental population and their progeny will be genetically homogeneous. The larger the number of segregating loci the larger will be the number of generations required to obtain a genetically homogeneous population. This will be true in the presence of polymorphism, as well.

Falconer[5], discussing the results of four selection experiments, says "the response continues for about 20 to 30 generations and the total range is between 15 and 30 times the square root of the additive variance . . .". Such a response cannot be explained by a small number of segregating loci. It has also been found that there is no appreciable change in the variance in the first few generations of selection. This favours the hypothesis of a large number of loci rather than that of a small number of loci.

There is, however, other evidence

which indicates that if the polygenic hypothesis did not exist it would have to be invented. Winter[6], for instance, selected from commercial varieties of corn, two diverging lines for high and low protein and two lines for high and low oil content. After years of selection experiments, the difference between the high and low lines for oil content was more than 20 times the original standard deviation. Discussing these results, Student[7] concluded that they could not be explained "on the basis of a few easily detected genes". He estimated the number at 100–300. Noting that there was no evidence that the selection had reached its limits, he concluded "that the number of genes affecting oil (or protein) content . . . may run up to thousands".

Fisher[4] was critical of Student's[7] estimate but felt that, in conjunction with other evidence, the results "... force one to the conclusion that all commercial varieties may be segregating in hundreds, and quite possibly, in thousands of factors influencing the normal development of a plant".

There seems to be no alternative hypothesis to account for these facts. Student[7,8] did suggest an hypothesis to explain Winter's[6] experiment, namely, that "species tend to accumulate a sufficient store of genes of no particular value until they meet with a change in environment when the store provides material for selection far beyond the normal range".This in no way diminishes the importance of the polygenic hypothesis; on the contrary, it strengthens it.

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THOMPSON REPLIES—Although I appreciate the position taken by Vetta[1] in defending the common hypothesis that quantitative variation is produced by the segregation of a large number of genes, much work has been done in the 30 or 40 years since Fisher, Winter, and 'Student' first considered the problem (see refs in Vetta[1]). Among the most significant recent contributions to this problem have been those by Thoday and his colleagues[2–5], particularly Spickett[6–8], and those by Scharloo[9] and Rendel[10]. It is well past time to reconsider our understanding of quantitative genetic traits, incorporating this information[11]. This is not to say that the older facts are necessarily wrong, but rather that our perspective of the problem should be reconsidered.

Genes affecting continuously distributed phenotypes have small effects relative to uncontrolled environmental variation, and allelic substitutions at individual loci are essentially equivalent. This often makes precise genetic analysis difficult. It is clear from biometrical literature that models based on assumptions of many genes having similar phenotypic effects can explain quantitative variation quite elegantly. But are these assumptions generally valid? What experimental evidence is there to support the assumptions (that is, experimental evidence which is independent of the statistical tests, themselves)? Are there equally satisfactory models based on other reasonable assumptions about the number or magnitude of polygene effects?

An alternative assumption, to which Vetta objects, concerns polygene number. Evidence comes from both theoretical and experimental sources and is summarised briefly in my original article[11]. One important observation is that theoretical distributions essentially indistinguishable from normal phenotypic distributions can be produced by segregation at a few loci, or even at a single locus. Several such examples are discussed in detail by Thoday and Thompson[12]. The conclusion is that there is no justification for automatically assuming that an apparently normal phenotypic distribution is caused by the segregation of a large number of genes. Experimental evidence confirms this[11].

Some, but by no means all, characters continue to respond to artificial selection for "20 to 30 generations". Vetta is quite correct, therefore, in pointing out that there are some traits for which it is likely that a comparatively large number of factors contributes to the variance. Indeed, I did not deny that this may often be true (ref. 11, p. 666). I suggested, however, that these are examples of highly complex characters (that is, they are the result of the interaction of a large number of contributing developmental processes) and that polygenic influences on each of these contributing processes would tend to be cumulative. Quantitative variation in body weight, for example, may be produced by polygenes affecting the rate of bone growth or the adult bone size, the efficiency of nutrient uptake from the intestine, endocrine function, the rate of cell division, cell number and size, the size of muscles, and numerous other contributing processes which, if looked at carefully, could each be considered quantitative characters in their own right. It is the analysis of these contributing processes which will give us the most rewarding insight into polygene number and function.

The point of contest should, therefore, not be whether there are "few" or "many" (at best, very unspecific terms), but whether the number of polygenes affecting any character is exceptionally large (the "hundreds" and "thousands" sometimes quoted). I believe that the balance of evidence provides no compelling reason to think that the number of polygenes affecting any particular trait is exceptionally large, and indeed, it is probably quite a lot smaller than generally thought.

The usual impression of multigenic effects is probably attributable, in many instances, to an unsophisticated approach to phenotypes and the processes of development. What is needed is an experimental approach to quantitative genetic variation which concentrates, not on the sheer number of factors[1], but on their functions and influences on development? The work by Spickett[7,8], Milkman[13] and others shows that this is potentially more informative, for often the number of genes involved is not particularly large, and in appropriate conditions they can be isolated and manipulated readily[2].

As long as continuously distributed phenotypes are regarded as necessarily the product of segregation at a large number of loci, straightforward chromosome substitution and other direct manipulations of the genome may not be given sufficient attention as possible alternatives to the more time-consuming and less precise techniques generally available to applied geneticists.

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reviews

Competition and community

David C. Culver

Ecology and Evolution of Communities. Edited by Martin L. Cody and Jared M. Diamond. Pp. xii+545 (Belknap for Harvard University: Cambridge, Massachusetts and London, 1975). $17.70.

THIS collection of 18 papers which grew out of a memorial symposium by former students and associates of the late Robert MacArthur contains a brief summary of his work and a bibliography of his papers. Continuing in the MacArthur tradition, the contributors look for and explain the broad patterns of community organisation, often at the expense of detail. A major theme running throughout is the impact of competition on community structure.

In the longest paper of the symposium Jared Diamond explains alternate stable communities of birds on islands near New Guinea by combining information about dispersal and competitive ability. Martin Cody investigates the diversity patterns of bird communities in Mediterranean climates and Henry Hespenheide assesses the importance of diet overlap in birds to species-packing problems. Using canonical correlation, James Karr and Frances James break new ground in identifying morphological and ecological correlates of bird communities. The negative correlation of bird species numbers with environmental variability suggests to John W. MacArthur that variability reflects the environmental resistance to invasion of new species. Taken together these studies provide strong support for the contention that competition is the major organising force in bird communities.

In papers dealing with non-avian communities—lizards (Eric Pianka), rodents (James Brown), butterflies (Arthur Shapiro) and diatoms (Ruth Patrick)—MacArthur's theories of species diversity seem less successful in explaining community structure. None of these papers directly confronts the basic premise that competition is the major kind of interspecific interaction. In general, predicted correlations match with the data but there are occasional bothersome discrepancies that either resist explanation or require elaborate qualifications to the theory. The pre-eminence of competition is challenged by Joseph Connell in a very provocative essay in which he argues that predation is much more important, except in harsh physical conditions. Connell proposes that experimental manipulations of communities yield more information than comparison of unperturbed communities. In another paper in this volume, Richard Levins shows, however, that the unexpected effects resulting from perturbations of complex communities can be difficult to interpret.

There are also several major theoretical contributions. Michael Rosenzweig develops several models which predict an asymptote to species diversity on continents. Henry Horn proposes a model of forest succession as a Markovian process which he partially tests with data from the Princeton woods. He also manages to include a few enjoyable barbs at critics of this kind of general approach to succession. William Schaffer and Madhav Gadgil develop models to explain life history strategies for plants, and Leigh develops some models to relate population fluctuations and species diversity. Robert May presents a major synthesis of the theoretical underpinnings of species abundance relations, and distinguishes the biological from the mathematical properties of such distributions. His work should facilitate the testing of hypotheses in this area. In a difficult but very important paper, Levins outlines loop analysis, a technique enabling the analysis of communities where the direction but not the magnitude of the effect of one species on another is known. He uses loop diagrams to predict some unexpected consequences (such as a species selecting itself to extinction) of evolution in complex communities. The two concluding papers look ahead—G. Evelyn Hutchinson reviews the relationship between theory and data in several areas of ecology, and Edward O. Wilson and Edwin Willis point out some possible applications of MacArthurian theory to the preservation of diversity in natural areas.

In general the symposium participants share a common view of ecology—one with an emphasis on rather imprecise models that predict overall patterns. In this volume the major weakness emerging from this approach lies in the too often sporadic and incomplete statistical testing of theory. That some authors seemed too committed to competition theory when consider alternative hypotheses led to its occasional reification. With the exception of the contributions of Levins and Hutchinson, there was no clear sense of the direction in which ecological theory should develop. This volume is a good summary, however, of the status of that part of ecology strongly influenced by MacArthur and his associates. ☐

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Monumental work revised

The History of the British Flora: A Factual Basis for Phytogeography. Second Edition. By Sir Harry Godwin. Pp. x+541+12 plates. (Cambridge University: Cambridge and London, 1975.) £30; $85.

THE publication of the second edition of Sir Harry Godwin's *History of the British Flora* has been an eagerly anticipated event in palaeoecological circles. There are few books which have had such a profound influence on the development of any branch of science than the first edition of this book. The volume of additional information which has accumulated since its publication in 1956 is, in large measure, a singular monument to its success. As a stand of reeds accumulates new material around itself, ultimately resulting in its own replacement, so has Godwin's work provided a basis for the research of many students, thereby encouraging the accretion of new records and findings. The appearance of a second edition of this book can therefore be regarded as the inevitable outcome of an autogenic process.

By far the most important revision of the book is the incorporation of the newly assembled data into the encyclopaedic list of Quaternary fossil records of each taxon. This section is preceded by a list of the sites and references to publications from which information has been gleaned—a list in which one can detect a degree of selectivity in operation. Space is used more economically than in the first edition, for no longer are the locations listed for all occurrences of the commoner fossils. Instead records are represented as frequency histograms in the various subdivisions of the Quaternary. Godwin retains his standard I–VIII zonation system for the Late-Devensian and Flandrian and it is difficult to see how else he could provide a systematic framework for the arrangement of plant records. A full discussion of the radiocarbon dates associated with these zones, however, would have proved most valuable. It is now known that many of the changes in pollen assemblages which are used as a basis for the Godwin zonation system are not synchronous (with the possible exception of the zone VIIa/VIIb boundary) across the country. But since the system has been widely used in the zonation of published pollen diagrams up to the time of the preparation of this book, it does provide the most obvious and widely understood method of presenting the data.

Godwin rightly claims that the fossil history of our flora provides a factual basis for the understanding of plant distribution patterns. It is also true that developments since 1956 have gone some of the way towards the unravelling of phytogeographical problems. The science is, nevertheless, still in its infancy and the scope for new ideas and methods is immense. New techniques are now being brought to bear on palaeoecological studies, many of which have been borrowed from related disciplines; these could provide new insights as well as new information concerning the history of the British Flora. Meanwhile this book presents a full and authoritative account of the present position of this subject.

If this edition is indeed the product of a successional process, it should not be regarded as the climax. Although the body of information contained within the book is impressive, it should be looked on as a progress report rather than as a concluding statement. The reeds are growing taller and the ground is almost firm enough to walk on, but the process of accretion will continue and may soon result in some profound and unexpected developments. Production rates are such that we may need a third edition before another twenty years have elapsed and I, for one, look forward to it.

Peter D. Moore

Valuable source of limnological data

A Treatise on Limnology. Vol. 3: Limnological Botany. By G. Evelyn Hutchinson. Pp x+660. (Wiley-Interscience: New York and London, February 1976.) $33.30; £16.75.

IN his preface Hutchinson promises the remaining limnological data he has assembled in smaller segments. Yet this third volume on limnological botany encompasses only chapters 27–32 and is 660 pages packed with information, but virtually excluding work on rivers. As readers of the previous two volumes will know his scholarship is impressive and his search of the literature most thorough from sixteenth century herbals of Dodoens to publications of 1974. As usual an index of lakes and of genera and species is included.

The first 70 pages are devoted to the rooted cryptogams which in lakes comprise only the Charophyceae and Bryophyta. These are relatively neglected components of the aquatic flora and the author has had to quote extensively from a few major works. Nevertheless he has highlighted several relatively unstudied aspects and problems—for example, the confused taxonomic situation in the Charophyceae, the need for greater ecological data on occurrence, zonation, germination of oospores, and so on. In passing, the odours produced by organic compounds in *Chara* and the larvicidal effects of Charophytes on mozquitoes are brought to our attention. The relationship between distribution in different water types and depth zonation of both Charophytes and Bryophytes is dealt with; but as Hutchinson comments on p. 63 most published records say little about ecology, and experimental studies especially of aquatic Bryophytes are scarce.

The next 438 pages are devoted to aquatic tracheophytes, a few of which are Pteridophytes *sensu lato;* but most are Angiosperms, of which 51 genera of Dicotyledones and 65 Monocotyledones occur in water. In the second chapter these are figured, and their distribution and life forms described. Although interesting, much of this purely botanical information will be of slight interest to limnologists—particularly the numerous categories of life forms culminating in a seven-page table. The third chapter concerning the biological characteristics of aquatic plants is a mine of information, including photosynthesis, carbon sources, heterophylly, development of aerenchyma (including figures of an Eocene aquatic fern) seed germination, pollination and dispersal (including that of the well known *Elodea*).

The fourth chapter concerns the uptake of nutrients, the chemical composition of the plant tissue and the distribution of the plants in relation to the chemical composition of the water. A large amount of data is assembled in this chapter, which forms the most comprehensive survey to date. The data on many chemical elements in water will be of interest, for example, to students of pollution. There then follows a chapter devoted to the depth distribution of macrophytes, the macrophytic flora of lakes selected from many regions, and a summary of the associations found in lakes based on phytosociological studies.

Finally the algal benthos in the widest sense (of all the algae, and briefly the lichens) associated with underwater surfaces) is summarised in a more adequate manner than in any other text, although here there is still a lack of data due to neglect of these communities in favour of the more easily studied plankton. The number of genera involved is very large and Hutchinson has not been able to survey these as comprehensively as those of the other freshwater associations.

There are only a few misprints, especially of authors names and some cross indexing without the page numbers. But otherwise this book is produced to the same high standard as the preceding volumes and alongside these it will remain an invaluable source for many years.

F. E. Round

Troubled waters

The North Sea: Challenge and Opportunity. (Report produced by Study Group of David Davies Memorial Institute of International Studies.) Pp. xiii+324. (Europa: London, 1975.) £6.95; $21.00.

SINCE the discovery of hydrocarbons at exploitable depths in the British sector of the North Sea, public policy towards the area has been dominated by considerations of energy and little else. On land, considerations of ecology and the environment have acquired sufficient political force for the formation of a single Department of the Environment. The concept of land-use planning has become part of the vocabulary of bureaucrats, politicians, and the media elite. The same cannot, alas, be said for the sea, especially for the North Sea.

Although voices have been raised—on the National Environmental Research Council (NERC), in the recent Fabian Tract on Sea Use Planning, at the various conferences organised by the Greenwich Forum, and in various submissions to parliamentary committees—little has changed. To quote from the above report:

> ". . different activities in the North Sea are regulated at different levels and with varying degrees of thoroughness . . . It is only where a problem or situation has been seen as critical that measures have been taken to try to meet it. Otherwise inertia and the usual short-term view that characterises most politics prevails Machinery is thus lacking for considering, evaluating and regulating for the long-term the complex of inter-related activities (which actually take place in the North Sea) . . . and this lack must be attributed to the absence of a general awareness of the need for such an integrated approach".

The report comments on the existence of a "widespread, if rather unfocused, realisation that there is a need for overall environmental planning. The danger, as always, is that lip service to an idea may become a substitute for hard thinking and eventual action; and this can postpone the recognition of the immediacy and gravity of the problem."

One of the most serious obstacles to such recognition in the past has been that those who recognise the "immediacy and gravity of the problem" in their own field are either scientists or technologists, lacking either the political understanding or the grasp of international issues necessary to convey their sense of urgency across those barriers to understanding or communication which divide the bulk of politicians, civil servants and leaders of opinion from the research laboratories, common rooms, technological seminars and institutes of engineering. The civil servant or politician, however, has as much trouble bringing home to the numerate the compulsions of international law, the resistance of vested interests, the difficulties of reaching a democratic consensus, and the nature of political realities.

One of the many merits of this study group is that it effectively married expertise in environmental science and in international law. The chapters on the international and national aspects of the present legal regime in the North Sea should be essential reading for all concerned with the scientific and technological sides of the exploration, exploitation and development of the resources of the North Sea. Equally compulsory reading for all specialists should be the chapter on the competing uses and interests in the North Sea.

The report points to various obvious *lacunae* in our understanding of the North Sea water budget; history of sedimentation; sea–air interaction; food chains, and basic chemical information on estuaries. To control man-made changes in the ecology of the North Sea very much more research needs to be undertaken.

Its recommendations on the political level are, to my knowledge, streets ahead of the thinking of any government policymaking body in Western Europe, calling at the national level for a considerable reorganisation of national administration, involving the establishment of a UK Cabinet Committee with responsibility for inter-departmental collaboration and of a permanent parliamentary committee on the marine environment: and on the international level for the establishment of a regional regime for the North Sea in the form of a Standing Conference of North Sea States with an international economic committee and a joint North Sea policing service. It will alas be some time before these become politically feasible. What we need now is a survey of the policies of the other North Sea states to match that dealing with Britain.

This book cannot be too strongly recommended, at a time when so much of Britain's future depends on the way in which the opportunities provided by the recent discoveries in the North Sea are realised.

D. C. Watt

Student physics

Introduction to Modern Theoretical Physics. by Edward G. Harris Vol. 1. Classical Physics and Relativity. Pp xvii+383+9. Vol 2 Quantum Theory and Statistical Physics. Pp. xvii+385–780+9. (Wiley–Interscience: New York and London, December 1975) £13.50, $27 00 each volume

PROFESSOR HARRIS remarks that the tendency to specialise has increased to such an extent that large gaps occur in the education of PhD candidates in physics He has attempted to write a textbook which covers everything such a student should know. The course covers mechanics, continuum mechanics, electromagnetism, relativity, general relativity, quantum mechanics, quantum field theory, elementary particles, thermodynamics, statistical mechanics and plasma physics.

The section on continuum mechanics is extremely short, whereas the treatment of general relativity is too ambitious (it includes attempts at unified field theory and geometrodynamics) The treatment of experimental questions in general relativity reflects the author's own enthusiasm, and in most subjects there is a balanced choice of topics The author even has time for a few historical remarks. Much of the book overlaps, intentionally, with undergraduate topics, even down to some things covered in the first year

Although the intentions of the book are good, I doubt that it will ever become the English *Handbuch der Physik.* The section on classical mechanics is less good than H. Goldstein's book, the quantum mechanics section would not be preferred to Dicke and Wittke; and so on—each topic is already well covered by existing texts at the same level.

The use of imaginary time $x_4=ict$ instead of the usual $x_0=ct$, leads to confusion between hyperbolic and elliptic equations. The author is over-cautious in condemning the Klein–Gordon equation as having "unsatisfactory features". The treatment of boundary conditions on the Schrodinger wave function is left vague with the statement that ψ should be "well-behaved". Understanding this point is not helped by his advocacy of the Dirac bra and ket formalism. A simple explanation is found in Kemble's book—one requires self-adjoint boundary conditions. The author is also clearly out of his depth (p. 400) when mentioning unbounded operators. I would have preferred the theory of the Dirac sea to have been left unsaid, its inconsistencies were bypassed by second quantisation 45 years ago.

Nevertheless, a physicist who knows everything in these books will be widely educated in modern theoretical physics.

R. F. Streater

Books brief

Astronomy: The Evolving Universe. By Michael Zeilnik Pp. xii+529. (Harper and Row: New York and London, 1976.) n.p.

THIS book will be jostling with about a dozen superficially similar texts for a slice of the American college market, since it is an integrated course aimed at students with an interest in astronomy. What differentiates it from its competitors are two features: it is up-to-date (about 1975), and it weaves the essential science around an intriguing theme, the evolution of the universe and the objects within it The classical ordering of material is fast falling into disuse as authors seek cosmetic devices to disguise the essential similarity of their books. This one certainly wears an attractive face.

Zeilnik's text spreads itself at great length across four major topics: man's changing ideas about the universe; the solar system; stars and galaxies; and cosmic evolution The test particles so dear to reviewers (black holes, pulsars, exobiology, nucleosynthesis, and quasars) are all here and explained with admirable clarity, along with massive doses of astronomical history, planetary science, and astrophysics.

I believe that this is a really outstanding teaching text for qualitative courses Every page is packed with facts, and the error rate is astonishingly low. Zeilnik clearly understands what writing for students is all about: his material is clear, interesting, correct and complete. Among the many new things I learned was that reading books printed entirely in royal-blue ink is very tiring. Otherwise, this is a fine effort that is certain to be adopted for general courses in the US and Canada.

Simon Mitton

Principles of Enzyme Kinetics. By Athel Cornish–Bowden. Pp. 206. (Butterworths: London and Boston, Massachussetts, February 1976.) £12

THE author of this work set himself the task of covering the major aspects of enzyme kinetics in a relatively small book, and the result of this extremely ambitious attempt is that the coverage of the different topics is rather uneven. At best the book is very good indeed and the accounts of the principles behind the schematic methods for deriving kinetic equations and the analysis of progress curves are clearly and logically presented. Some of the other sections are, however, less satisfactory. In the chapters on fast reactions and on the effects of pH and temperature, for example, the author tends to select aspects of the subjects for short comments without providing any detailed account of the general principles involved in the analysis and interpretation of experimental results. In addition the condensed nature of the book leads to the author making a number of categorical statements without any justification, some of which might be regarded as being arguable by others working in the field. For anyone with a reasonable knowledge of enzyme kinetics this book should offer a number of interesting insights, but the patchy coverage does not recommend it as an introductory textbook for the subject.

In spite of its limitations I found the book to be extremely valuable for its excellent treatment of statistical methods for evaluating kinetic results. This is a subject that has received scant attention in the past and the lucid account presented here should be of great help to anyone attempting to interpret the results of kinetic experiments. **Keith Tipton**

Antimetabolites of Nucleic Acid Metabolism: The Biochemical Basis of Their Action, with Special Reference to Their Application in Cancer Therapy. By Peter Langen. Pp xii+273. (Gordon and Breach: New York, London and Paris, December 1975.) £10.

THIS is a rather dull, very condensed and encyclopaedic account of the nature and mode of action of a wide range of anti-metabolites affecting the biosynthesis and function of nucleic acids. It is not a readable book, partly because of the prose style, but also because of the type face used and the somewhat inadequate legends to many of the illustrations.

The first five chapters deal with historical and general features of anti-metabolites, mode of action, acquired resistance, degradation and biochemical aspects of cancer chemotherapy. Each chapter opens with some general considerations before proceeding to illustrate these with specific and detailed reference to particular compounds.

The second part of the book tends to be something of a catalogue of anti-metabolites, some of which receive substantial treatment, whereas others are dealt with very briefly indeed.

In the foreword, the authors indicate that this new edition contains much new material, but it is a pity that some of this has been dealt with in an addendum rather than being integrated into the text.

As it stands, the book contains a great deal of information and a very extensive bibliography. It will certainly be of value to research workers in this field by drawing together information and ideas from widely scattered sources, but it is a book for a reference library rather than for the individual purchaser. It is unfortunate that it does not include a detailed alphabetical index.

R. M. S. Smellie

Organic Reaction Mechanisms—1974. (An annual survey.) Edited by A. R. Butler and M. J. Perkins. Pp. 659. (Wiley-Interscience: London, New York, Sydney and Toronto, February 1976.) £25.00; $55.00.

THIS volume, produced by two new editors, follows closely on the pattern set by its predecessors—in itself an excellent recommendation. The period covered is December 1973 to November 1974 and being the tenth volume in the series it incorporates the second quinquennial index. The subdivision of the subject matter has not been changed substantially—reactions of aldehydes and ketones and their derivatives, including enzymic reactions (B. Capon), reactions of acids and their derivatives again including sections of enzymic catalysis and reactions (M. I. Page), radical reactions (D. C. Nonhebel and J. C. Walton), oxidation and reduction (T. W. Bentley), carbenes and nitrenes (M. S. Baird), nucleophilic aromatic substitution including sections on Meisenheimer complexes and benzynes (M. R Crampton), electrophilic aromatic substitution (B. V. Smith), carbonium ions (R. Baker), nucleophilic aliphatic substitution (I. D. R. Stevens), carbanions and electrophilic–aliphatic substitution (R. B. Boar), elimination reactions, I, polar addition (A. C. Knipe), addition reactions, II, cyclo-addition (W. E. Watts) and molecular rearrangements (A. R. Butler, G. V. Meehan and M. J. Perkins). Each chapter is clearly subdivided, amply illustrated by formulae, fully referenced, and the overall format is of a high standard. The price of the volume is very high even allowing for the detailed content, but this series is a must for all well-stocked libraries of organic chemistry. **A. W. Johnson**

nature June 17, 1976

On location with the JET set

FUSION power is unlikely to contribute significantly to Britain's energy resources over the next 50 years. But its energy potential is so large that development of the technology must be continued: That view from ACORD, the Advisory Council on Research and Development for Fuel and Power, is contained in a discussion document, *Energy R&D in the United Kingdom* (other details, see page 535), which accords the subject barely a couple of paragraphs. But it does remark that there is general scientific agreement that the next step in the nuclear fusion programme should be the Joint European Torus (JET). And that is the rub. Everyone in Europe, not just Britain, probably agrees what the next step should be. But they differ sharply on where it should be.

What Mark Twain said about the weather, in fact, is not inappropriate to Europe's decision on a site for JET: everyone talks about it, but no one does anything about it. The long dispute has become altogether too protracted to make any sense. In February, the council of Research Ministers again postponed the decision they had already put off last December, when the new five-year European fusion programme involving JET was to be finalised. They managed agreement on initial financing of the project, but when it came to JET's location, the European Commission and Italy plumped for the European Joint Research Centre at Ispra while Britain lobbied for Culham (the design team's base), France for Cadarache and West Germany for Garching.

In an effort to reach a decision, a "fusion consultative committee" was formed, chiefly to re-examine the alternative sites but also to fulfil a more general advisory role. It was to report to the next Research Ministers' meeting, due this week (June 18). When it met in April, the committee said a decision to build JET was needed forthwith. But it did not deal with the question of the site, even though it was thought it might give its verdict early in May at a Foreign Ministers' meeting—the level at which the issue might need resolution. The agenda for the Ministers' meeting, however, was too full to include JET. By the time the consultative committee was due to meet again in May, the Italian election was in prospect and the story was already about that, again, no decision would be taken. Now the June 18 meeting itself has been postponed.

This tale of procrastination invites exasperation and despair, not least on the part of the design team. It is understood that a formula, including financial arrangements, is now being sought which might go some way towards maintaining the team's cohesion. It is to be hoped that this materialises. Members of the team had two-year contracts which expired last December and which were renewed for six months on the now-frustrated expectation that a decision would have been reached. That did not prevent the departure of some of them then. It is not certain that it will do so now. But every effort must be made to keep these people together if Europe is legitimately to imagine itself serious about nuclear fusion.

On that ground alone an early decision seems vital. The technical assessment of each of the alternative sites is not likely to change significantly: some are better than others in some respects, others are better in other respects. There is a balance of judgment involved which it is difficult to strike because it is so fine. But the decision is essentially a political one anyway. There is no gainsaying that already complex problems are complicated further by this fact. But things have now reached a crucial stage. A healthy element of competition in scientific research is essential to progress. And that progress is only possible in the fusion field on a European scale. The danger presented by the delay over JET now threatens the prospects for nuclear fusion as a future energy source by undermining Europe's hitherto prominent position in this field in comparison with the USA and USSR.

Few people are yet saying that the USA and USSR should be allowed simply to get on with it. Those countries themselves want Europe in on the venture anyway. But there are many who, by scrambling so unedifyingly over what is admittedly a real plum and one of the most lucrative research projects of the decade, are allowing Europe's case to go by default. The Nine must now ask themselves: if the politics of petty nationalism is impairing European co-operation on fusion to the extent that no decision on a site for the project can be made, can the people of Europe really be offered much hope for the aims of the project itself? The institutions of Europe may not reveal it, but the people they serve so indirectly are the ones who are ultimately paying for the present mess. And the cost, both financial and otherwise, is growing all the time. □

The search for a Law of the Sea

THE coastal states of the world, in particular those with wide continental shelves, have for ten years now been working on certain principles: what we can grab by inventing new rules, we will claim for ourselves; what's left can be designated as a common heritage of mankind; and the profits therefrom (if there are any) can be shared amongst States Parties to the Convention (including ourselves) but (as a sop) "taking into particular consideration the interests and the needs of the developing countries". Their object has now nearly been achieved, and it looks as if we shall see in the next year or two a Convention that allocates to the coastal states a 200-mile economic zone plus almost certainly the resources of the continental margin where that margin extends beyond 200 miles. So far as scientific research is concerned, its fate within these zones will be barely distinguishable from that in the Territorial Sea, as it will be tightly controlled by a "consent" regime throughout.

What is left is known as the "Area" —the sea-bed and ocean floor and subsoil thereof (that is, not the water column) beyond the limits of national jurisdiction. These limits of national jurisdiction have not yet been defined; a number of proposals have been made, but what is common to all is that the limits are in deep water, thus ensuring that virtually all exploitable areas are kept firmly within the national jurisdiction of the coastal states.

The United Nations is becoming more adept with each decade at developing white elephants, but the possibilities opening up now as a result of the deliberations of the Third UN Conference on the Law of the Sea are even wider.

To control and supervise exploitation of the mineral sources of the Area, there will be an International Sea-bed Authority which has the potential of developing a secretariat several hundred strong with "regional centres or offices" and all the trappings of a UN specialised agency. Added to this, the Authority will have an Enterprise to carry out its activities in the Area. This Enterprise will have a "Board of Governors, a Director-General and such other officers and staff to perform such duties as the Enterprise may determine".

The task of the Authority and its Enterprise will be to extract and market mineral resources from that part of the earth's crust that is physically most difficult, and economically most costly, to exploit, with the proviso that in so doing they must not upset the world's money markets in these commodities. As there is little likelihood of the Authority and its Enterprise becoming a profit making concern in the foreseeable future, here is a golden opportunity for the main financing member states of the United Nations and its specialised agencies to pour millions of dollars into an activity of doubtful economic or social relevance for legal-political reasons, instead of into international marine science, food programmes, fisheries and so on, which are being systematically starved of funds in the present world economic state.

The main working document of the conference is known as the "Single Negotiating Text". This first appeared at the end of the third (Geneva) session in mid-1975 and has now been revised as a result of "negotiations" during the fourth (New York) session. The size of the task before the conference (and the UN secretariat) can be realised when it is seen that the latest version of this Single Negotiating Text contains 398 Articles, as well as a number of annexes covering all aspects of the work of the conference. Many of these articles are not acceptable to countries or groups of countries, particularly the landlocked or geographically disadvantaged states which have been very active in asserting their "rights" during the recent session. They are therefore subject to further negotiation. Furthermore, a number of articles are incompatible with others developed in different committees and will need to be reconciled.

One of the mysteries of the conference has been the continued insistence of certain countries on the need to distinguish between fundamental and resource oriented research. This is a completely valueless exercise which in the long run can only do more harm to its promoters, through loss of belief in their honesty and intentions and in goodwill, than a policy of accepting Macmillan's "Winds of Change" and realising that going half way to meet the other man will pay dividends in the long run. The tactics of using a false assumption, one known to be false by the other side, as an item for negotiation are extremely doubtful. The developing countries, the policies of which are formulated by politicians notoriously only concerned with short-term results, and with no tradition of pure research, not surprisingly are unable to believe that developed-country insistence on this point is not a cover for activities which will be detrimental to them, either militarily or commercially. Quite apart from the impossibility of formulating a true distinction, their experience of publicised episodes of this kind, even if in fact minimal in comparison with genuine oceanographic research expeditions, does not augur well for the next so-called negotiating phase of the conference.

The most important outstanding difficulties now appear to be the status of the economic zone, the regime for the deep sea-bed and subsoil and dispute settlement procedures. The USA, which is putting pressure on the conference to reach a conclusion, and whose delegation will be led at the next session by Dr Kissinger himself, has called the arrangements for dispute settlement "the glue of the whole treaty", stating that "without agreed and binding dispute settlement procedures, the treaty will not be adequate".

The President of the conference, Mr H. Shirley Amerasinghe of Sri Lanka, has proposed that the first two or three weeks of the fifth session be used for making attempts to reconcile outstanding differences on the most crucial issues involved in drafting the Convention, followed by indicative but not binding voting in the committee on the various articles in the Single Negotiating Text. Certain delegations have objected to these proposals and it is too early yet to know how the matter will be handled. There is a feeling on many sides that if the conference does not achieve a reasonable consensus by the end of the next session, it will collapse, and no Convention will be possible for many years to come. Should this happen, the consequences would be endless and could well lead to severe clashes between states on unilaterally imposed limits and restrictions which would almost inevitably be to the disadvantage of the smaller and developing coastal states, owing to their physical inability to patrol and police a 12-mile territorial sea, let alone an economic zone 200 miles or more wide

The conference was sharply divided on the desirability of holding the next session with only a short recess, in order to keep up the momentum generated in New York, or as a further annual session in Spring 1977. In the event, after an appeal from the President, it was decided to hold the fifth session, from August 2 to September 17, again in UN headquarters, New York. It is hopefully envisaged that this will be the final working session and that it will be followed later in the year, or in early 1977, by the plenipotentiary Conference in Caracas to conclude the Treaty. □

Lithium and nuclear fusion

Can lithium reserves sustain a programme for the generation of power by controlled thermonuclear fusion?

Nick Walton and **Ed Spooner** offer this assessment

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THE postponements by the EEC of a decision on the siting of the Joint European Torus (JET) nuclear fusion project, worrying setbacks though these are for European fusion research, should not be allowed to detract from other considerations regarding fusion power and, specifically, questions about ore reserves of lithium and future lithium demand.

Of the three possible practical methods for obtaining power from fusion, the one based on the deuterium–tritium reaction is the most favourable since the "ignition" temperature is the lowest (about 10 keV) and the average energy gain is the highest (about 1,800 per fusion reaction). The first generation of nuclear fusion reactors will therefore need a continuous supply of both deuterium and tritium fuel.

Deuterium constitutes 0.015 atom % of naturally occurring hydrogen, and is relatively inexpensive to produce from sea water. Since sea water contains about 33 g m^{-3} deuterium, the world's resources of deuterium are large (about 5×10^{13} tonnes) and readily available Tritium, however, is an unstable isotope with a short half-life of 12.3 years, and occurs naturally only in trivial amounts as a product of cosmic ray bombardment in the upper atmosphere. A continuous supply of tritium for use in fusion reactors will, therefore, have to be produced artificially The only practical method of doing this is to irradiate lithium with neutrons:

$${}^6Li + n \rightarrow {}^3H(T) + {}^4He + 4.8\ \text{MeV}$$

$${}^7Li + n \rightarrow {}^3H(T) + {}^4He + n' - 2.5\ \text{MeV}$$

In a fusion reactor liquid lithium may be arranged as a circulating blanket surrounding the reacting plasma. Neutrons released by the fusion reactions can then be used to breed tritium fuel continuously, actually within this blanket.

The most useful of the two reactions is that involving ^{6}Li, since this is exothermic and can add 4.8 MeV per nuclear reaction to the energy output of the deuterium–tritium fusion process itself The capture cross-section of this reaction, however, peaks for slow neutrons at 0.3 MeV; it would thus use only a small proportion of the total neutrons available, since the spectrum of the neutron flux peaks at 14.1 MeV. The ^{7}Li reaction is therefore important, even though it is endothermic, because in addition to producing tritium it acts as a moderator of fast neutrons since its capture cross-section peaks at about 8 MeV. This reaction slows fast neutrons down sufficiently for them to interact exothermically with ^{6}Li and produce more tritium.

A blend of ^{6}Li and ^{7}Li can therefore balance the opposing requirements of tritium breeding and production of additional energy. The isotopic composition of natural lithium (7.4% ^{6}Li; 92.6% ^{7}Li) corresponds closely to an optimum mixture; it is worth noting, however, that the tritium required by the first nuclear fusion reactors will have to be produced by irradiating concentrated ^{6}Li with the slow neutrons available from fission reactors. Additionally, liquid lithium is theoretically superior to liquid sodium as a coolant. It combines such favourable properties as a low density, a high boiling point, a high heat capacity and a high heat transfer coefficient. Hence, an equally important factor which is likely to ensure that lithium will be used in nuclear fusion reactors is that it is theoretically highly suitable for use as the coolant which transfers heat from reactor cores.

Natural lithium in the form of a liquid metal blanket will, therefore, probably be essential in nuclear fusion reactors since it alone can effectively combine the three diverse requirements of tritium breeding, neutron moderation and heat transfer. Total cumulative Western world lithium demand up to the year 2030 may conveniently be considered as the sum of four requirements, each of which can be evaluated separately (see Table 1): non-fusion use up to the year 2000; non-fusion use from 2000 to 2030; capital requirements of lithium to be used in multi-purpose blankets for fusion reactors; and lithium consumption by nuclear reactions in the liquid metal blankets of fusion reactors.

Non-fusion demand

Western world consumption of lithium in 1974 was about 3,900 tonnes metal. Demand for lithium, which is used principally as an additive to aluminium potlines, in the manufacture of glass, ceramics, greases and specialised lithium chemicals, and in welding and brazing fluxes, increased at about 5% per year in the 1960s and early 1970s. However, the unique properties of lithium are only now being exploited for highly specialised, non-substitutable applications in a variety of new and expanding industries. This has resulted in a sharp rise to a 15% annual growth rate in the last few years, a rate which is likely to continue for the immediate future at least. Over the past 65 years annual growth in demand in the United States has fluctuated considerably in response to changing economic circumstances, but has averaged about 10%.

Two major new applications of lithium can be foreseen which are likely to sustain a high rate of growth in demand. These are in high power/weight ratio batteries, and as the liquid metal coolant in fast breeder nuclear fission reactors Annual growth in demand for lithium over the next 25 years is thus likely to be between 5%

Table 1 Estimates of Western world non-fusion and fusion demand for lithium up to the year 2030

(a) Western world lithium consumption in 1974[1]: 3,900 tonnes metal

(b) Non-fusion demand up to the year 2000:

Growth rate in demand	5%	10%	15%
Annual demand in 2000 (tonnes)	13,900	46,500	147,600
Cumulative demand by 2000 (tonnes)	200,000	426,000	958,000

(c) Non-fusion demand from 2000 to 2030, and cumulative non-fusion demand up to the year 2030:

Demand growth rate up to 2000	5%	10%	15%
Demand growth rate from 2000 to 2030	5%	10%	5%
Annual demand in 2030 (tonnes)	60,000	811,000	638,000
Cumulative demand between 2000 and 2030 (tonnes)	923,000	7.6 million	9.8 million
Total cumulative demand up to 2030 (tonnes)	1.1 million	8.1 million	10.8 million

(d) Capital requirements of lithium needed in fusion reactors from 2000 to 2030: ~1.5 million tonnes

(e) Lithium consumption by nuclear reactions in liquid metal blankets from 2000 to 2030: ~80,000 tonnes

(f) Total Western world cumulative demand for lithium by the year 2030: 2.7–12.4 million tonnes and 15%. Continual development of new applications is likely to sustain a higher rather than a lower rate, and this will lead to a cumulative demand for lithium by the year 2000 of between 200,000 and 958,000 tonnes metal (Table 1). The rate of increase in demand for the years 2000 to 2030 is also likely to be between 5% and 15%. The figures suggest that cumulative Western world non-fusion demand for lithium should be between 1.1 and 10.8 million tonnes. Because of the discovery of quantitatively important new applications for lithium, it is considered that estimates in the upper part of the range are more reasonable.

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[1] Luckenbach, W F., *Mining Annual Review* **1975**, 112 (Mining Journal, London; 1975)

Fusion demand

Future requirements of lithium for nuclear fusion reactors can be divided into two types: an initial inventory of lithium which will be used in the cooling blankets—this is estimated (Ribe, F. L., *Reviews of Modern Physics,* **47,** 7–41; 1975) to need between 600 and 1,700 tonnes per 3 GW reactor; and a supply of lithium which will be used to replace the lithium destroyed by the nuclear reactions in the liquid metal cooling blankets (it is considered that this will be a relatively small quantity of about 13.5 tonnes per year per 3 GW reactor).

In order to estimate the quantity of lithium which will be needed if a nuclear fusion programme does develop, a model calculation is used which is based on a set of postulates. These are:

- Commercial nuclear fusion reactors will start to be built in about 25 years time For technical reasons the power generative capacity of these machines is likely to be in the 2–5 GW range, and, therefore, larger than the current largest power stations which produce only about 1 GW.
- The rate of installation of nuclear fusion reactors between 2000 and 2030 will be comparable to the planned rate of installation of fission reactors in the United States between 1970 and 2000. A lower and more likely figure for the planned amount of generating capacity to be installed by 2000 of 750 GW is taken, rather than the more normally quoted figure of 1,000 GW.
- The Western European countries, and possibly other nations, will also implement a nuclear fusion programme which will together be approximately the same size as that in the United States.

Hence, assuming a mean fission reactor size of about 1 GW and a comparable building rate for fission and fusion reactors, approximately 750 fusion reactors might be installed in the United States between 2000 and 2030. At about 1,000 tonnes of lithium per fusion reactor, this would lead to a cumulative demand for lithium of 750,000 tonnes by the year 2030. If usage in the rest of the Western world is comparable, then cumulative Western world demand by 2030 would be about 1.5 million tonnes. This figure is comparable to the minimum estimate for cumulative non-fusion demand (1.1 million tonnes, Table 1). Cumulative demand for lithium to replenish liquid metal cooling blankets would be about 80,000 tonnes.

The total cumulative Western world demand for lithium up to the year 2030 could therefore lie between 3 and 12 million tonnes, depending principally on the growth rate of non-fusion uses.

Lithium occurrence and reserves

The concentration of lithium in the Earth's crust is of the order of 20 ppm, and in sea water about 0.2 ppm. Because of the small size of its univalent cation (ionic radius of $Li^+=0.68$ Å), lithium is an incompatible element which concentrates in the late-stage silicate melt and aqueous fluid phases formed during solidification of magmas. Crystallisation from these residual liquids has produced the unusually coarse grained acid igneous rocks called pegmatites. However, less than 10% of all known pegmatites contain suites of rare minerals, and only some of these are rich in lithium.

Table 2 Western world identified resoures of lithium

Country	Location	Type of deposit	Total identified resources (tonnes Li)[1]	Identified resources[2]	
				Proven and probable reserves (tonnes Li)	Possible additional reserves (tonnes Li)
USA	Kings Mountain, N. Carolina	Large pegmatites	313,000	441,000	560,000[3]
USA	Black Hills, S. Dakota	Small pegmatites	not specified		10,000
USA	Other areas	Small pegmatites	not specified		1,000
USA	Silver Peak, Nevada	Underground brine (300 ppm Li)	553,000	454,000	2,000,000
USA	Searles Lake, California	Surface brine (70 ppm Li)	40,000	10,000	30,000
USA	Great Salt Lake, Utah	Surface brine (60 ppm Li)	707,000	not specified	not specified
USA	Salton Sea, California	Underground geothermal brine (210 ppm Li)	1,000,000		1,000,000
USA total (rounded)			**2,600,000**	**900,000**	**3,000,000**
Canada	Barante, Quebec	Medium pegmatites	86,000	76,000	
Canada	Bernic Lake, Manitoba	Medium pegmatites	73,000	41,000	
Canada	Other areas	Pegmatites	317,000		180,000
Canada total (rounded)			**476,000**	**117,000**	**180,000**
S.W. Africa	Walvis Bay	Small pegmatites	17,800	4,000	
Rhodesia	Bikita tinfield	Medium pegmatites	83,900	82,000	
Zaire	Manono and Kittdo	Large pegmatites	1,000,000 (inferred)		1,000,000[3]
Africa total (rounded)			**1,100,000**	**86,000**	**1,000,000**
W. Australia	Coolgardie	Small pegmatites	15,000	not specified	not specified
S. America	Salar de Atacama	Underground brine	1,300,000		1,000,000[3]
Western world total (rounded)			**5,000,000**	**1,100,000**	**5,800,000**
				7,000,000	

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[1] Norton, J. J., *USGS Professional Paper*, **820,** 365–378; 1972.

[2] Kunosz, I. A., in *Industrial Minerals and Rocks* (AIME, 1974).

[3] These figures are hypothetical resources that are geographically predictable as existing in known districts.

The clinopyroxene mineral spodumene ($LiAlSi_2O_6$) is the most important lithium mineral. It occurs particularly in a small number of large unzoned pegmatites in North America. Lepidolite mica ($KLi_2Al(Si_4O_{10})(F,OH)_2$), eucryptite ($LiAlSiO_4$), petalite ($LiAlSi_4O_{10}$) and the phosphate amblygonite ($LiAl(PO_4)(F,OH)$) are the only other lithium minerals of economic importance. These minerals tend to occur as replacements in the central zones of smaller, zoned pegmatites which occur in Southern Africa and elsewhere.

Lithium also occurs concentrated in certain surface and underground brines. For example, the underground brines at Silver Peak, Nevada contain about 300 ppm Li, the Great Salt Lake, Utah contains about 60 ppm Li and the Salton Sea geothermal underground brine in California contains about 210 ppm Li. The Silver Peak brine is the largest known single reserve of lithium in the world and contains identified possible reserves of 2 million tonnes lithium metal. Lithium supply has recently become dominated by production from brines, and is likely to remain so in the future.

Unlike sodium and potassium, lithium does not occur in economically significant concentrations in marine evaporites because the concentration of lithium in sea water is low (about 0.2 ppm) and the solubilities of its salts are high.

For comparison with the estimates of cumulative Western world demand for lithium by the year 2030 given in Table 1, information on resources of lithium in economically workable concentrations (ore and brine deposits) is summarised in Table 2. This suggests that Western world reserves of lithium which are identified at the present time are about 5–7 million tonnes of metal. The amount of lithium in undiscovered deposits would add a considerable, but unknown, amount to this figure. In the past there has not been a large demand for lithium and, as a result, exploration for new deposits has not been particularly rigorous or extensive. The likelihood of discovering new large lithium ore deposits, therefore, is reasonably strong.

The figure of 5–7 million tonnes for identified Western world lithium reserves is comparable to the estimate of 3–12 million tonnes for Western world cumulative lithium demand up to the year 2030. Providing that exploration for new lithium-enriched brines and ore deposits is as successful as would seem probable, it can be concluded that the Western world's resources of lithium are capable of sustaining a programme for the generation of power by controlled thermonuclear fusion. However, the fact that the figures for cumulative demand and presently known sources are similar suggests that there is no cause for the complacency shown in recent assessments (see, for example, Hubbert, M. K., *Scientific American*, **225**, 61–70; 1971, and Proceedings of the Fourth International Conference on the peaceful uses of atomic energy (Geneva) **7**, 467 (UN and IAEA, Geneva; 1972)). This is particularly the case for the European countries which possess no presently identified significant lithium resources. This fact suggests a necessity for exploration in Europe if dependence on imports is to be minimised.

Four other conclusions may be drawn:

- Non-fusion demand for lithium is likely to be greater than or equal to fusion demand.
- Demand for lithium is certain to rise, and, therefore, lithium production will have to increase quite considerably. If, for example, the total growth rate in demand were maintained as high as 10% over the next fifty-five years, as over the past fifty-five years, production capacity would have to increase by a factor of about 200.
- A large amount of liquid lithium metal must be tied up in the blanket of each new fusion plant. This represents a sudden, large increased demand equal to one-quarter of current annual consumption. This capital demand for lithium has invariably been overlooked when assessing the availability of lithium supplies for nuclear fusion.
- The future demand for lithium will be relatively inelastic since the important new uses being developed are all based upon specific and unique properties of the metal. There are, therefore, no known lithium substitutes which could significantly depress projected growth figures. □

UK ENERGY

A question of balance

The widely heralded public debate on the national energy strategy opens next Tuesday, one week after publication of the Department of Energy's report singling out the coal and nuclear industries as crucial factors in the country's energy future. Allan Piper reports

ON the premise that the best way of planning a forward march is to stand well back and view the terrain ahead, Britain's Energy Secretary, Mr Anthony Wedgwood Benn, has started mapping out the nation's energy strategy with his feet in the right place. Consider the position. The country is caught in industrial recession, and inflation is at an uncomfortable level. The demand for energy has slumped. The coal industry, committed to an ambitious expansion plan after years in decline, is producing more fuel than the country can burn, and is staggering beneath the expensive burden of record stockpiles. The nuclear programme, years behind target and way over budget, is beset by niggling technological teething troubles, and faces powerful environmentalist opposition. Additional competitive pressures stem from the rosy opportunity for national energy sufficiency offered by North Sea oil and gas. But both must be carried through to the 1990s, when North Sea reserves will be running down and high world prices make energy imports undesirable.

It all means that when Mr Benn moves onwards from next Tuesday he will be juggling as he goes. Clutching the medium-term North Sea opportunities firmly in one hand, and manipulating nuclear options with the other, he must all the while keep the coal industry safely off the ground. Neither can he afford to drop any of the five realistic alternative possibilities—solar, wave, wind, tidal and geothermal power. Fusion is regarded as too distant. Thus, by the time the energy gap looms, the whole act must be sufficiently well coordinated to allow just one firm step across.

Events of the past fortnight have shown that the Energy Secretary will be jostled from every side. In the build up to the coming energy forum, organisations as diverse as the coal industry, the UK Atomic Energy Authority (UKAEA) and the Confederation of British Industry (CBI) on the one hand, and Friends of the Earth (FOE), consumer groups, and trades unions on the other, have deluged his department with a welter of initial positioning statements. They indicate clearly that few of the 120 participants involved will fail to adopt attitudes largely of self interest.

ACORD report

The simultaneous publication of a long-awaited Department of Energy (DEN) report on energy research and development (R&D) strategy may help, however, to keep the energy secretary on a firm footing. Its recommendations, while arguably as predictable as those of the various positioning papers, are at least based on wider considerations. Put together by a working group of the Advisory Council on Research and

Development (ACORD), the report identifies coal and nuclear technologies, together with energy conservation, as outstanding long term R&D priorities. At the same time, it calls for the priority development of technologies to optimise the medium-term benefits of North Sea resources.

The report's conclusions are not based on the conventional method of forecasting distant energy demands, by extrapolating historical trends into the future. Instead, the ACORD working group has considered seven possible "scenarios" and suggested strategies considered necessary to meet the challenges of each one. The scenarios range from a continuation of present social, economic and technical trends until 2025, through a pattern of low worldwide economic growth, to one of high worldwide growth.

The picture that emerges suggests that whichever scenario prevails Britain is unlikely to get by comfortably without well developed coal and nuclear technologies. Even with a continuation of present trends, the report finds, the UK's installed nuclear capacity must rise to almost 25 times the existing level of 5,000 MW by 2010 if substantial energy imports are to be avoided. This is so even though the coal industry should by then have reached its expansion target of 150 million tonnes a year (25 million tonnes above present levels), since the likely decline of North Sea reserves will shift coal's greatest economic contribution to its potential as a source of gas, liquid fuels, and other hydrocarbons.

If the analyses are correct—and ACORD must feel reasonably assured of a wide response to the report's invitation for alternative scenarios from independent interests—the renewed emphasis on the crucial importance of the coal and nuclear options raises Mr Benn's greatest problem. As Walter Marshall, Chairman of ACORD and Chief Scientist at the DEN, put it last week, there is a danger that complacency in the face of adequate medium term North Sea supplies could delay major investment decisions until it is too late to retrieve the situation.

Coal

The coal industry's own solution to this thorny problem became available last week with the publication of a positioning statement for the energy debate from the National Coal Board (NCB) and coal workers' unions. It shows that the industry wants the Central Electricity Generating Board (CEGB), coal's biggest single customer, to review forward orders for coal fired or dual-fired generating plant. But as the CEGB Chairman, Arthur Hawkins, pointed out when the issue was first raised in February, his utility can hardly pull the plug out of the North Sea market, nor the rug from under the nuclear industry, which between them should eventually supply the constant CEGB demand baseload.

Moreover, the CEGB has already given some ground to help relieve the coal industry's position. Under a payment deferral scheme it has agreed to hold coal stocks in excess of normal reserves—put at 50 days supply—so that it is now stockpiling nearly 17 million of the nation's total 31 million tonnes, a figure expected to have reached 20 million tonnes by the end of September. To offset this increase, at least in part, the CEGB will take advantage of the recent slight improvement in coal's economic competitiveness against imported oil to burn 70 million tonnes this year, an improvement of 3 million tonnes over last year's level. If the short-term investment hurdle can be cleared, the coal industry will argue, the way is open towards the targets for the future. Estimated UK reserves are put at around 45,000 million tonnes, enough to last for several centuries at current extraction rates, and the industry claims that future demand will be more than matched by production capacity.

Thus, it will be reasoned, a national energy strategy based on coal imports would be misguided. During the 1980s indigenous coal could resolve the burden of imported oil costs, and even, with the introduction of fluidised bed combustion and an increased uptake by the home steel industry, Britain could become a coal exporter as early as 1985. As to the less immediate future, the industry, like ACORD, foresees its greatest long-term economic potential in gasification and liquefaction techniques. NCB R&D spending into these fields and into integrated and remote mining systems is planned at £12 millions for this year.

Nuclear

The nuclear industry stands just as firmly behind its own position. Stressing its "excellent" safety record, the UKAEA will argue that because coal's greatest potential lies where it does there should be no major national commitment to its short term use as a generating fuel, and that the nuclear option provides the only realistic long term alternative that can be available in time and in sufficient quantity. To meet future demands, the UKAEA says, an independent R&D effort should be coordinated within the industry. The UKAEA also calls for some stability in the forward ordering pattern for power stations, while the Nuclear Power Company claims that orders must run at more than 1,000 MW a year if a rundown in construction and design work is to be avoided.

Walter Marshall: Britain's energy guru?

Recent developments on the nuclear front suggest that the CEGB might view this plea for increased nuclear capacity more favourably than it might have done even a few weeks back, in spite of its continuing pessimism over future levels of electricity demand. Last month it was announced that the troublesome Advanced Gas Cooled Reactor at Hinkley Point in Somerset is now producing Britain's cheapest electricity, at about one eighth of the cost of the most efficient fossil-fuelled station at Ratcliffe-on-Soar. Almost simultaneously, the Hinkley Point reactor obtained the long-overdue go-ahead to run up to heavy loads, and it should soon have reached 85% (about 475 MW) of designed operational output. The second reactor at Hinkley Point is also expected to come on stream soon, and it seems possible that the AGR at Hunterston on the Scottish coast could produce usable power before the winter.

Furthermore, Dr Marshall is ready to present a report on nuclear pressure vessels to the Chief Nuclear Inspector, concluding that they could safely operate in Britain without major design changes. While the finding provides an immediate fillip only to Westinghouse Electric, the US company responsible for the design and sales of the Pressurised Water Reactor, it will nonetheless indirectly boost national confidence in nuclear technology.

The CEGB may also be impressed by the ACORD analysis of the future role of nuclear power, as may Mr Benn. This indicates primarily that whichever future economic scenario is considered, an enlarged nuclear capacity seems virtually essential. The only non-nuclear scenario, that excluding nuclear plant beyond the 1980s, throws up a gloomy picture of economic growth restricted to "unacceptably low levels".

Fast Breeder

Should these favourable developments

lead to the general acceptance of plans for an enlarged nuclear technology, the vexed question of long-term uranium availability will certainly raise calls for the early introduction of the Fast Breeder Reactor (FBR), which uses uranium 60 times more efficiently than conventional reactors. The ACORD report does not consider the possible effects of an international moratorium on the FBR, but the oversight is something that the increasingly respectable Friends of the Earth, averse to the FBR in particular rather than nuclear reactors in general, may seek to amend. A government decision on whether to move from the prototype to the demonstration stage with the FBR is to be given in the autumn, and it is still possible that Britain could forego further independent development, opting instead to buy herself into other national programmes at a later stage. The CEGB is known to be engaged independently in ongoing negotiations with the French-German-Italian consortium involved in the Superphénix FBR.

Conservation

On conservation, there is general accord, with calls from both the nuclear and coal industries for tightly controlled exploitation of North Sea resources. This would not only stretch oil and natural gas reserves, but should also provide the coal and nuclear programmes with a chance to regain a firm grip on the generating market. British Gas, however, will tell the Energy Secretary next week that such measures are largely unnecessary, suggesting that further gas finds could close the predicted energy gap completely.

But, more broadly, ACORD has underlined that because of low energy conservation factors, which average around 40%, every unit of power conserved represents more than two units of primary energy. This is a proportion which may become critical in the energy climate of the late 1980s, and last week Dr Marshall announced that the portion of the DEN R&D budget set aside for conservation—included, incidentally, with the allocation for alternative resources—is to be expanded by over £7 millions to £22 millions within the next four years.

For its part, the CBI will call for increased consumer influence in energy affairs, a theme also taken up by the National Consumer Council. On the other side of the political fence most of the unions can be expected to underline the already published call of the Amalgamated Union of Engineering Workers for complete nationalisation of North Sea resources. The AUEW also decries any extension of oil and gas use into electricity generation.

So it all begins on Tuesday with the shouting. Some may doubtless regard it as fitting that the Energy Secretary should launch his juggling act under a glare of national publicity. Others more charitably feel that if the right attitudes win through the event could be just the start of an effective exercise in "open government". Whatever the outcome of his eclectic approach one thing is abundanty clear: Mr Benn will need a cool head and steady eye to strike the necessary balance. ☐

NUCLEAR TRADE

Heavy water allegations

The controversy over international trade in nuclear technology and materials continues in the United States. Colin Norman reports from Washington on the latest development

THE long-simmering debate over nuclear export policies took an important new twist in Washington last week. Senator Abraham Ribicoff, chairman of the Senate Government Operations Committee, charged in a public statement that heavy water supplied by the United States to India in 1956 played a key role in India's nuclear explosives programme. Ribicoff, an outspoken critic of present US nuclear export controls, sharply criticised the State Department for failing to place strict safeguards on the heavy water sale, and he also condemned the department for failing to establish whether India used the material to produce its explosive device.

Though the State Department and the Energy Research and Development Administration (ERDA) have challenged the chief point in Ribicoff's analysis, his statement is likely to play an important part in public hearings on whether or not the United States should sell some 40,000 pounds of enriched uranium to India to fuel a large nuclear power plant near Bombay. The hearings have been postponed from June 2 to July 20.

According to Ribicoff, the United States sold India 21 tons of heavy water as a moderator for the Canadian-supplied CIRUS reactor, the source of plutonium for India's nuclear explosive. The material was supplied under an agreement that it be used only for peaceful purposes, and Indian officials have claimed that the explosive device detonated in May 1974 was produced without the need to resort to imported material.

The Indian assertion rests on the argument that heavy water in the CIRUS reactor degrades by about 10% a year, so that all the US-supplied material would have been used up and replaced by India's own heavy water (produced in a German-supplied plant) well before its explosives programme was begun. That argument was accepted by State Department and ERDA officials, who reiterated last week that they have no reason to doubt its validity. But Ribicoff claims that loss of heavy water in the reactor is much less than 10% a year, and that a substantial amount of US-supplied material was in the reactor while it was being used to manufacture plutonium.

Whatever the validity of Ribicoff's claims, the suspicion that US-supplied heavy water helped India to produce nuclear explosives is likely to be influential in next month's hearings on the sale of reactor fuel to India. Though the reactor in question, the Tarapur Atomic Reactor, is operated under safeguards administered by the International Atomic Energy Agency (IAEA), critics of the proposed sale have argued that India should accept additional safeguards on its nuclear facilities before final approval is given to the deal.

The IAEA safeguards are designed to prevent plutonium produced in the Tarapur reactor from being used to manufacture explosives. If the United States refuses to supply fuel for the reactor, however, it is conceivable that India could claim that since its nuclear agreement with the United States had been broken, safeguards on the plutonium no longer apply. According to Ribicoff, the Indian Government may be using that possibility as a "subtle form of blackmail" to ensure that the fuel sale is approved, and he argued that the United States "should seek to end the present uncomfortable and uncertain situation by exercising an option we have to buy back the plutonium generated by these power reactors".

At least one of Ribicoff's claims is, however, incorrect. He argued that the United States "never publicly acknowledged exporting the heavy water to India", but ironically, the Atomic Energy Commission trumpeted the 1956 agreement in a press release since it was one of the first nuclear deals under President Eisenhower's Atoms for Peace Programme. ☐

AUSTRALIA

Major appointments at stake

Peter Pockley reports from Sydney on the important jobs now open on the Australian science scene

WHILE the organisational structure of Australian government-financed science and advisory machinery is still being sorted out, the government has the opportunity of making some even more significant changes through the clean sweep of senior science appointments which have to be made between now and the middle of next year. The jobs at stake are the Chairmanship of CSIRO, the Chairmanship of the Australian Atomic Energy Commission (AAEC), and the Permanent Head (Secretary) of the Department of Science. Between them, these positions control over $200 million of funds, well over the total of all other government research and development funds put together. Appointments to the three positions are in the gift of the government, formally by different ministers, but in practice the Prime Minister, Mr Malcolm Fraser, is expected to take a strong hand in all selections.

Dr Jerry Price, the Chairman of CSIRO, is now in his last year of office; he will reach the retiring age of 65 next March. A committee to examine the filling of the vacancy has been appointed, reportedly by Mr Fraser himself even though CSIRO reports to the Minister of Science, Senator James Webster. The committee is operating very privately, but is understood to be chaired by a leading academic. Not only has it been charged with recommending a prescription for the ideal candidate, but it is also looking critically at the structure of the CSIRO Executive and its decision-making processes.

Sir Hugh Ennor: retiring early?

CSIRO operates as a statutory authority under its own Act of Parliament, the Science and Industry Act. The Executive is the body responsible under the legislation for CSIRO's activities, and much interest attaches to appointments to the Executive and its terms of reference. The Labor Government had determined last year to allow the staff of CSIRO to elect one of the part-time members of the Executive, but the Liberals snuffed out the staff's hopes in this regard by getting elected to Government just before the election process had gone into the period of no-return. The part-timer whose retirement from the Executive was creating the vacancy for a staff member has had his term extended for a short period by the Liberal Government, thereby avoiding the need for a decision on the politically sensitive question of formal staff involvement on the boards of statutory authorities.

The CSIRO staff are making their views known to the committee on this and other matters of contention within the organisation. One proposal emerging is the possibility of restructuring the 9-member Executive (Chairman, 4 full-timers, 4 part-timers) into a "troika" of Chairman and two full-timers concerned with broad policy issues and an "outer" executive charged with administrative functions. The key to any major changes is the appointment of the Chairman. Dr Price was promoted to the Chairmanship from the Executive in 1970; this time the field is probably wide open, including to outsiders.

Sir Hugh Ennor, Secretary of the Department of Science, will reach retiring age of 65 in October 1977. Like many senior Australian Government public servants over 60, though, there will shortly be a prospect for earlier retirement with the introduction of a new superannuation scheme in July. With the Department he established in 1972 currently under threat of abolition, Sir Hugh Ennor can be expected to fight to go out with full Secretary status. Absorption of his Department within another one before his retirement would be a bitter pill, and Sir Hugh is not a man to be lightly put down.

Nonetheless, a decision will have to be made within a year about who is to head the administration of the operational branches and sections presently within the Department of Science, whether the Department exists as a separate entity or, for example, is recombined with the Department of Education. This appointment is highly likely to go to a senior public servant. Mr Fraser has set himself against outside appointments to permanent headships following his inheritance of some of Mr Whitlam's appointees, two of whom are still languishing on the so-called "unattached list" where they draw their nice salaries and do nothing while awaiting appointments which never come.

The Chairmanship of the AAEC is vacant because of the sudden death on the job earlier in the year of Mr Bill Boswell, who had been full-time Chairman since 1972. The AAEC has been left in a very uncertain position, since both the Labor and the Liberal Governments had failed to appoint Commissioners to fill two part-time vacancies in the Commission of five. With Mr Boswell's death, the AAEC is left with only two part-timers. The previous Vice-Chairman, Sir Lenox Hewitt, is filling in as Acting Chairman while also holding down his full-time post as Chairman of Qantas. Professor Harry Messel, the other part-timer, a strong contender for the Chairmanship given more time under Labor, is probably now a non-starter on grounds of having been too closely associated with the former Labor Minister, Mr Rex Connor. If so, the appointment will have to come from outside.

The Minister for National Resources responsible for the AAEC is the Leader of the Country Party and Deputy Prime Minister, Mr Doug Anthony. A strong man who will want to appoint his own AAEC Chairman, Mr Anthony will nevertheless have to defer to Mr Fraser because of the Prime Minister's newly won influence over the whole science scene through the Australian Science and Technology Council which reports direct to him. While ASTEC does not have appointments advice within its charter, its membership does contain one or two likely contenders for the CSIRO or AAEC jobs.

Overseas aspirants, please note: the public servant pecking order in Canberra is determined largely by relative salaries, but there are some other interesting comparisons. The Chairman of AAEC currently receives 31,000 Australian dollars. Next is the Chairman of CSIRO on only $32,500 ("only" because the CSIRO budget is roughly 10 times that of AAEC). The Secretary of the Department of Science receives $35,000, for running an operation about a third of CSIRO's in terms of money and staff. ☐

● *Stop press*: Professor Donald W. George, Vice-Chancellor of the University of Newcastle, has been appointed part-time Chairman of the AAEC. The other two AAEC vacancies have also been filled.

IN BRIEF

California split

Voters in California rejected, by a margin of two to one, a proposition which would seriously have impeded—and possibly have halted—the use of nuclear power in California. The outcome was greatly influenced by the last-minute approval of three nuclear safety bills spawned by the debate over the proposition. Governor Edmund Brown signed them into law a few days before the vote, a fact which enabled him to demonstrate his concern for safety without the risk of alienating voters by coming out in favour of the proposition.

No visit from USSR

Academician Yakov Zeldovich, winner of three Lenin Prizes and one Stalin (now State) prize for physics, was unable to attend a ceremony at Cambridge last week at which he was to receive an honorary DSc degree. There is speculation that the Soviet authorities refused him a visa for security reasons. Yet earlier in his career Academician Zeldovich was closely connected with defence work, including research into the physics of conventional and nuclear explosions, but was not prevented from visiting London in 1965 to attend the Fourth International Conference on Relativity and Gravitation.

Of mites and moles

KENNETH MELLANBY

Do research workers know why they choose a particular subject to study? Today there are those who say that they have no right to make such a choice. It is suggested that they should rely on some "customer", backed by a committee or other collection of wise men, coming forward and telling them what they should do, and even how they should do it. While the working of this system can hardly be said to have contributed to scientific productivity, it is perhaps timely to look at the way in which those who have had the choice have exercised it. Has it generally given better results?

Several times in my career I have been fortunate enough to be able to choose my own research problems. During the 1939–45 War I was adequately supported by a rather unrestrictive research fellowship, while I was temporarily barred from military service by the register of reserved occupations. I felt that my somewhat academic studies in insect physiology had little relevance to the war, so I searched for a more practical problem. Having contacts with medical entomology, I soon found that the parasitic disease scabies, or "the itch", was causing increasing concern. Soldiers as well as civilians were suffering and were immobilised by treatment. It was also feared that any breakdown in national hygiene following enemy bombing might precipitate an uncontrollable epidemic. The disease was caused by the tiny mite, *Sarcoptes*, burrowing into the skin, and it was clear that a better knowledge of its biology was needed. I have, until recently, assumed that I decided to work on scabies for humanitarian and patriotic reasons; now I am beginning to re-examine my motives.

A later opportunity for choice occurred some ten years ago, when I was director of a research station. Provided that the station ran fairly smoothly, the staff got on with their work, and I showed up at an adequate number of committee meetings, no one objected if I did a little research in the remainder of my time. There would have been difficulties if I had chosen an expensive field and asked for a lot of money for equipment, but I needed to show that my view that good work could be done cheaply had some foundation. So I decided to work on the mole, *Talpa europeanea*. At the time I believed that I made this choice because I thought that the mole could be a simple indicator of the potential fertility of the land it infested. Text books told us that moles were very voracious, eating twice their own weight of earthworms daily. As worms abound in largest numbers in fertile soil, I hoped that a count of molehills could suggest whether the ground was or was not worth cultivating. Unfortunately many of the well-known "facts" about moles proved to be wrong, their appetite had been overestimated, and the largest number of hills sometimes appeared in the poorest soil. The mole cannot be used to indicate fertility; but it is still a fascinating creature.

Recently, I have found myself once more involved with these two problems, scabies and the mole. At first sight they appear to have little in common, one concerning a medical pest, the other a minor pest of agriculture. I have just returned from a conference on scabies in Minneapolis sponsored by the American Medical Association, the American Academy of Dermatology and other bodies. In order to give three lectures on the biology of the *Sarcoptes* I had to re-read my publications of over 30 years ago and check the results with recent observations. It was gratifying to find that the subject had remained virtually unchanged for this period. At about the same time as I was setting off for America a children's book which I had just written on the life of a mole appeared. I found myself comparing these two apparently very different creatures.

The itch mite is a tiny eight-legged arthropod, the mole is a warm-blooded mammal. Their main resemblance is that they both burrow, the itch mite in the human skin, the mole in the soil which could be described as the world's epidermis. The mite's burrowing causes a disease of the skin; gardeners whose lawns have suffered may think the mole gives rise to similar symptoms. But the burrowing mode of life can cause remarkable similarities in very different creatures. Both mites and moles are solitary, remaining in their tunnels for all or most of their lives. Both feed in the burrow. The young are born in the burrows, and then migrate out and eventually start their own tunnels. The males are more mobile than the females, they actively search for mates, but they separate again after copulation.

Having made this comparison, I began to examine my original motivation. Were the admirable and socially acceptable reasons I originally put forward for these studies the correct ones? Or have I some deep seated psychological urge to study the phenomenon of burrowing? In my case both investigations were reasonably productive. Should all scientists undergo psychological examinations, to fit their problems to their subconscious predilections? Perhaps research workers, to be successful, need to deceive themselves regarding their real reasons for choosing a subject, as well as those who they approach for financial support for their investigations.

correspondence

Selling the Sun

Sir,—Although man's existence depends, and has always depended, on nuclear fusion, the only functional system so far devised is some millions of miles removed and its major benefits are brought to us via photosynthesis. The "land issue" which should be faced is the fact that most surfaces are already covered by solar cells. These are called "green plants" and, at the molecular level, they can convert light energy into chemical energy with an efficiency of about 30%. Because most crops will not grow throughout the year or make best use of the available light, the realisable maximum is about 5% and in much agricultural practice may be of the order of only 0.1%. Nevertheless we are entirely dependent on this miserable conversion rate (and on past photosynthesis in the shape of fossil fuels). Is it then so far removed from reality and responsibility to suggest that we spend a little more than we do on increasing the effectiveness of biological solar energy conversion?

If terrestrial nuclear fusion becomes a reality it may make a massive contribution to our energy requirements. Photosynthesis (itself only one small item in the very large report on solar energy) does this already. Moreover, when all our oil and coal is spent it will have to serve as the major source of the elaborated carbon which we presently use unchanged or in the production of iron, plastics, fabrics and so on.

Money spent on nuclear fusion may be money well spent, but practical success is not guaranteed. Conversely, biological solar energy conversion has been with us for a long time At the practical level it is hopelessly inefficient but there is no life without it. The room for improvement is immense. May it not even command a fraction of the expenditure on nuclear fusion?

Yours faithfully,

D. A. Walker

Department of Botany,
University of Sheffield,
Sheffield, UK

Spraying controversy

Sir,— In the article by David Spurgeon entitled "Spraying controversy revived" (May 20, page 184), it is suggested that emulsifiers used in insecticide sprays used for budworm control may be linked with Reye's syndrome.

If this is confirmed, the reference to the condition being first described in 1963 following the US Air Force's aerial defoliation in Thailand and Vietnam will need to be examined critically since none of the three herbicidal agents used for defoliation in Vietnam contained emulsifiers.

Details are available in the report of the National Academy of Sciences on the "Effects of herbicides in South Vietnam", published in 1974. It may be relevant to add that this very full report contains no reference to Reye's syndrome.

Yours faithfully,

J. D. Fryer

Weed Research Organisation,
Yarnton,
Oxford, UK

Alternative refrigerants

Sir,—W J. Megaw's letter (May 6, page 10) contributes to oversimplification concerning the fluorocarbon industry, and thus may mislead the incautious reader.

Refrigerants *leak* from refrigeration units. The causes are recognised engineering factors such as shaft seals, maintenance operations, permeation and eventual scrappage. Refrigerant half-life ranges from about 4 years for mobile and large fixed installations (non-hermetically sealed) to about 12 years for the hermetically-sealed units. The latter type is largely confined to small domestic units for sound engineering reasons.

Once a refrigerant cycle and capacity have been specified, the selection of refrigerant is made. R-22 is used where its physical, chemical and thermodynamic properties are suitable, but it is not well-suited for all uses. For this reason, R-22 accounts for only 30% of refrigerant sales rather than 50%, as suggested by Megaw. Forcing its use as a refrigerant across the board would result in major design and performance problems.

R-22 and R-12 can be manufactured in the same plant only if the plant was originally designed as a multi-purpose unit. Most fluorocarbon plants are dedicated to the manufacture of products derived from a common starting material. This approach provides the most efficient use of materials, energy and manpower, and results in the lowest-cost product.

Industry and government-supported research is under way to get the facts in this environmental question. Recent technical developments stemming from stratospheric HCl measurements have forced further reductions in calculated ozone depletion. With these results, there is no reason at this time to implement Megaw's recommendation to sacrifice personal care products.

We all realise that our environmental decisions should be based on facts rather than emotion. Wrong "facts", however confidently presented, are not adequate.

Yours faithfully,

Richard B. Ward

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Water to the Dead Sea

Sir,—It has been suggested in your columns that both electric power (November 6, 1975, page 9) and water (February 12, 1976, page 444) could easily be generated from the flow of seawater into the valley of the Dead Sea (surface at −390 m). Besides using this head for turbine generated energy, both additional turbine and electrical energy could possibly be obtained from the osmotic volume flow across a permselective hyperfiltration membrane and from the so-called dialytic battery (*Science*, **191**, 557; 1976) respectively.

Limitations to the above methods should however, be kept in mind. These include: the loss of energy due to friction and volumetric flow due to evaporation; the practical need to operate seawater hyperfiltration at about 2½ times (or more) the osmotic pressure of the feed (that is, 72.5 bar or 725 m of water); the problems of concentration polarisation (salt build-up) at the membrane solution interface reducing the effective driving force; and the fact that brackish water is available near the Dead Sea and could possibly be demineralized at a lower price than seawater.

Thus, the proposed scheme to generate electric power and potable water for the arid Dead Sea region should be evaluated in light of the above limitations.

Yours faithfully,

Georges Belfort

Human Environmental Sciences Program,
School of Applied Science and Technology,
Hebrew University,
Jerusalem, Israel

news and views

New finds from Lake Turkana

from a Correspondent

ONE of the most exciting aspects of anthropological discoveries in East Africa over the past few years has been the clear demonstration of the existence of the genus *Homo* at a date much earlier than previously thought. Before these discoveries some palaeoanthropologists had advocated a model of human evolution centring on a single, slowly evolving lineage. Although the evidence in Bed I, Olduvai Gorge and at Swartkrans, South Africa, had earlier indicated two separate, contemporaneous forms of hominid, the "single species hypothesis" continued to find favour in some circles. The discovery of KNM ER 1470 in 1972 was a crucial event in effectively challenging the one lineage concept. Subsequently, evidence of the early presence of *Homo* has been reinforced by further discoveries at Lake Turkana (formerly Lake Rudolf) and at Hadar, Ethiopia. Preliminary reports on Mary Leakey's new material from Laetolil, near Olduvai Gorge, may place the earliest evidence for the genus there at about 3.75 Myr. The lowest levels at Hadar, which may contain *Homo*, may also be near that date.

It now seems clear that the genus *Homo* existed at a very early date. It also seems clear that the taxon *H. erectus*, generally agreed to be ancestral to *Homo sapiens*, may have more antiquity than previously realised. In this issue of *Nature* (page 574), Richard Leakey reports the discovery of a new skull from Lake Turkana (KNM ER 3733); this skull bears a number of similarities with material from Peking which has been attributed to *Homo erectus*. A pelvis (KNM ER 3228), discovered stratigraphically below the skull, shows some features like those found in OH 28, from Bed IV, Olduvai Gorge. OH 28 has also been attributed to *Homo erectus*.

While the newly described material from Lake Turkana is not the earliest evidence of *Homo erectus* it may be the earliest yet found in Africa. Previously, the earliest clear African member of *Homo erectus* was the OH 9 skull from near the top of Bed II, Olduvai Gorge; this level has a date of about 1.1 Myr. The taxon has, however, been reported from the Djetis beds, in Java, in levels probably slightly younger than 1.9 Myr (Jacob, *Antiquity*, **47**; 1972). Although the data of the new Lake Turkana material is not clear it would seem to have a minimum date of 1.3 Myr (Leakey and Walker, page 572 of this issue).

This strong evidence of the early and widespread presence of *Homo* poses some interesting questions. Foremost among these perhaps is the nature of the evolutionary relationships between this genus and known members of the genus *Australopithecus*. Although the classification of this Plio–Pleistocene genus is the subject of some controversy, it is generally agreed that within this group was a heavily built, "robust", lineage probably demonstrating some dietary specialisations. This group, variously called *Paranthropus robustus*, *Australopithecus robustus* or *Australopithecus boisei*, was in clear sympatry with *Homo* at several African sites There is little agreement, however, on whether other Plio–Pleistocene hominids of a less robust morphology should also be placed within this genus. Some workers do recognise a "gracile" species, *Australopithecus africanus*, best known from Sterkfontein, in South Africa. Others, such as Robinson, however, have postulated that all members of the "gracile" type should be placed within the genus *Homo*. It now seems possible that such a two-lineage model, like the one-lineage model, is untenable. A prime species of evidence against the inclusion of all gracile hominids within *Homo* is AL 288, the associated cranial and post-cranial remains recently reported from Hadar, Ethiopia (Johanson and Taieb, *Nature*, **260**, 293; 1976). This particular individual has several characters not usually included within generic definitions of *Homo*. For example, the third mandibular premolar has two cusps of very unequal size. In other hominids the premolars usually have two cusps of nearly equal size, although the third premolar in the Fort Ternan mandible (KNM FT 45), usually referred to as *Ramapithecus*, has a condition somewhat similar to that seen in AL 288. Moreover, the femur of AL 288 has the flattened neck and lack of flare in the trochanteric region usually considered characteristic of australopithecine femora. Johanson and Taieb have in fact suggested attribution of this individual to *A. africanus*.

Such sympatry of *Homo* with one, and quite possibly two, forms of australopithecine would mean that there is little information about the direct and immediate ancestors of *Homo*. These known australopithecines, contemporary with *Homo*, obviously cannot fulfil the ancestral role. Finally, however, the coexistence of possibly three Plio–Pleistocene hominid groups may indicate, indirectly, something about the very nature of still earlier hominid evolution. When organisms initially occupy a new habitat they often experience a period of rapid adaptive radiation, so that eventually they occupy a variety of "niches" within that new habitat. During their Cainozoic history the order Primates have, in fact, experienced several vigorous radiations. It is possible that the early hominids, too, went through a period of adaptive radiation when they first began occupying the forest margin, grassland habitats in the Miocene and early Pliocene. It is possible that here, in the early Pleistocene, we are seeing the fossil evidence of such a radiation.

A hundred years ago

WE regret to hear that the strife at Sidney about the dismissal of Mr. Krefft from the post of Curator and Secretary of the Australian Museum is not over. The subject came before the Legislative Assembly on the 6th of April, and provoked an angry discussion. Mr. E. P. Ramsay has been installed by the trustees as Mr. Krefft's successor, and is in full work; but the Supreme Court has decided that the trustees had no real authority to remove Mr. Krefft.

from *Nature*, **14**, June 15, 157; 1876.

Cosmic X-ray bursts

from Kenneth Brecher

COSMIC X-ray bursts, first observed by the Russian Cosmos 428 satellite in 1971 and reported in a widely overlooked paper by O. P. Babushkina *et al.* (*Sov. Astron. Lett.*, **1**, 32; 1975) have now been detected by many other X-ray satellites. The ANS, SAS 3, Vela 5, and Ariel V groups have reported in rapid fire succession a range of exciting and perplexing observations of these fast transient cosmic events. In this issue of *Nature*, (page 562) J. Heise *et al.* report on their observations of "rapidly repetitive X-ray burster" MXB 1730−335 made with the Astronomical Netherlands Satellite. This object, discovered by the Massachusetts Institute of Technology SAS 3 group and reported by Professor Walter Lewin (*IAU Circ.*, 2922, March 5, 1976) is perhaps the most enigmatic "X-ray burster" found to date. Sometimes bursting at several-second intervals, at other times waiting minutes between events, its X-ray burst pattern reminds one very much of a relaxation oscillator. Indeed, as pointed out by Professor George Clark (MIT) the tight correlation found in the SAS 3 data between the X-ray burst size and the time to the following burst is reminiscent of the behaviour of a neon light bulb (rather than, for example, a flush toilet). It is as if a reservoir of energy (or material to provide it) must build up to a fixed level and discharge; the larger the discharge, the longer it takes to build up for the next firing. (In the flush toilet analogue, with filling occurring at a constant rate, the size of a burst would be proportional to the time preceding it.)

What astrophysical object could produce such behaviour? The ANS group report a position for the X-ray burster which they intersect with the SAS 3 error box: the overlap region contains what according to W. Liller (Harvard College Observatory) may be a globular cluster (a stellar system several light years across consisting of 10^4–10^6 stars). The importance of the possible association of the rapid burster with a globular cluster cannot be overemphasised: it may be something specific to these stellar systems as a whole, rather than the individual stars within them, which gives rise to the observed X-ray bursts. Heise *et al.* point out that at least three bursters appear to be associated with globular clusters. Nonetheless, it should be noted that at least four burst sources reported by the MIT SAS 3 group—MXB 1728−34, a source in the Galactic anti-centre region, one in Puppis, and another in Aquilla (*IAU Circ.*, 2922, March 5, 1976, and private communications from W. Lewin)—are not obviously associated with globular clusters, or, if they are, there is no clear reason why the optical counterparts are so obscured as to be utterly unobservable. It is truly remarkable that of the 100 or so Galactic globular clusters known in the halo population the unobscured ones are not X-ray bursters, but that it is claimed that several of the obscured ones are. Perhaps a second hitherto unsuspected disk population of obscured globular clusters, or ones containing invisible stars, houses these bursters.

Making the bold assumption that it is in the nature of globular clusters to burst out occasionally, what is it specifically that gives rise to the X-ray bursts? It has been suggested for some time that a globular cluster could become unstable to the formation of a very dense or tightly bound stellar configuration at its centre. Taking this view one step further, J. Bahcall and J. Ostriker (Princeton University) and, independently, J. Arons and J. Silk (University of California, Berkeley) proposed that the steady X-ray emission observed from five globular clusters arises from accretion of intracluster gas on to a central, massive (10^2–$10^3 M_\odot$) black hole. Adapting rapidly to the new observations, Bahcall and Ostriker have now suggested that these same black holes can also emit X-ray bursts, provided that a neutron star were orbiting the hole. Postulating an accretion disk of gas around the black hole, the neutron star would then periodically crash through the disk, slurp up some gas, and belch out the observed X-rays. This model, offered as an explanation for the recurrent X-ray burst source apparently associated with the steady X-ray source 3U1820−30 and possibly located at the centre of the globular cluster NGC6624, is difficult to reconcile with the very brief time interval between bursts from the rapid burster. Indeed, the change in mean burst period from 0.18 d (Clark, *IAU Circ.*, 2907) to 0.12 d (Clark, *IAU Circ.*, 2932) makes this model suspect even for NGC6624. The suggestion by J. Grindlay and H. Gursky (Center for Astrophysics) that the pulse shape of the X-ray bursts could arise from the scattering of a sharp X-ray pulse on a spherical cloud of very hot gas (rather than a disk) which is held down by a massive black hole is at variance with the above suggestion. As shown in detailed Monte Carlo calculations by C. Canizares (MIT), however, the shape of the decay phase of the X-ray bursts can be well described by Compton scattering of the photons on cooler gas surrounding an object of a few solar masses rather than a massive black hole. Unless one devises one model for each source, a procedure which seems premature in view of the remarkable similarity in pulse structure from one burst source to another, one must turn from black holes to some other, more physical and realistic, model.

Most optically identified discrete Galactic X-ray sources found to date have been associated with mass exchange binary star systems, probably containing as one member a collapsed object (neutron star, white dwarf, or black hole). Various authors, such as J. Arons and S. Lea (Berkeley), have suggested that instabilities in accretion flow on to the collapsed object could give rise to rapidly variable X-ray fluxes from these objects. W. Baan (MIT) has suggested that accretion disk instabilities occurring around single neutron stars accumulating gas while moving through globular clusters could account for many of the particular properties observed in MXB 1730−335. Flash nuclear burning on the surface of a neutron star has been proposed as an X-ray burst model by L. Maraschi (Milan) and A. Cavaliere (Frascati). Other models, such as the stellar lightning model offered by Rrose Selavy (Zurich) have not been worked out in sufficient detail to make a direct comparison with observations possible at present.

Despite the fact that in the past few years black holes have been offered up as a panacea for explaining everything from the Tunguska event, to the absence of solar neutrinos, to the energy emitted by quasars, and even as a solution to the terrestrial energy crisis, there seems to be little direct observational support for the assertion that X-ray bursters provide evidence for the existence of massive black holes. If it turns out that the X-ray bursts arise as the result of one more complication in mass exchanging binary star systems, they will almost certainly lose some of their current fascination and mystery. Still, it must be said that it is really too early to tell whether the X-ray bursts are the astronomical discovery of the decade, or ffiust a flash in the pan. ☐

Venus could have a large magnetic moment

from Peter J. Smith

TEN to fifteen years ago the only planets thought to possess internal magnetic fields were the Earth and Jupiter. But the data provided by space probes have transformed this picture—so much so that planetary fields must now be regarded as the rule rather than the exception. The strongest known field is still that of Jupiter which has a magnetic moment of 1.4×10^{30} gauss cm^3. Then comes the Earth with a moment of 8×10^{25} gauss cm^3, Mercury with 5×10^{22} gauss cm^3 and Mars with 2.5×10^{22} gauss cm^3. The Moon (admittedly not a planet), on the other hand, has no detectable internal dipole, which means to say that if it has an internal field at all the moment must be lower than 10^{19} gauss cm^3.

The absence of a lunar field is explicable on the basis of the Moon's small core and its low rate of rotation. More surprising, however, especially in view of the fields discovered on the other terrestrial planets, is the apparent lack of magnetism on Venus. According to Dolginov *et al.* (*Kosmich. Issled,* **7,** 747; 1969) the upper limit of a barely detectable Venusian moment must be 5×10^{21}–8×10^{21} gauss cm^3. Yet on the grounds of size alone Venus would be expected to have a core about as large as that of the Earth (and thus larger than that of Mercury), and its rate of rotation is only about four times lower than that of Mercury. On this basis Venus ought to have a moment of 1.9×10^{23} gauss cm^3, or so a forthcoming report predicts. Why, then, has a magnetic field greater than the Dolginov limit not been observed?

The first attempt to measure the global field of Venus was by Mariner 2 in 1962. No field was detected at the closest approach of 6.6 radii—a result which placed an upper limit of 4×10^{24} gauss cm^3 on the Venusian moment. Five years later the flyby of the USA's Mariner 5 apparently enabled this limit to be reduced to 8×10^{22} gauss cm^3, a figure derived from the observed position of the bow shock. But in the same year (1967) Russia's Venera 4, which landed on the planet's surface, measured the magnetic field directly, down to an altitude of 200 km. It was these data, combined with those from Mariner 5, which Dolginov and his colleagues used to reduce the maximum possible moment to 5×10^{21}–8×10^{21} gauss cm^3, the lowest upper limit yet proposed.

But Russell (*Geophys. Res. Lett.,* **3,** 125; 1976) now claims that an alternative interpretation of the Venera 4 data is possible. Limiting his analysis to the last 10 min of the field measurements (representing altitudes of less than 5,000 km), Russell shows that for three different models of the solar wind–Venus interaction there is a strong dependence of magnetic field on altitude, with the field increasing in strength towards the planetary surface. Moreover, for two of these models the altitude dependence is close to an inverse cube law, the exception to this being the least likely model which assumes no well-developed magnetosphere. In both cases the extrapolated surface field is about 30 nT, which would be equivalent to a magnetic moment of 6.5×10^{22} gauss cm^3.

In short, the altitude dependence is consistent with the existence of a planetary field, and the magnitude of the field suggests that the internal moment, if there is one, is larger than that of either Mars or Mercury. The qualification is needed here, however, because the form of the Venera 4 data, though necessary, is not sufficient to prove the presence of an internal global field. There are alternative interpretations. The magnetic observations could, for example, be explained not only in terms of an internal field but also of a field induced in the ionosphere. The rejection of one or other of the options will probably have to await further data, preferably from a Venus orbiter. But in the meantime a Venusian moment of at least 10^{22} gauss cm^3 must be regarded as at least plausible and, if Venus is not to be exceptional, even likely. □

Genu valgum, copper and fluorosis in India

from Zaka Imam

FIRST reported in 1973 (Krishnamachari and Kamala Krishnaswamy, *Lancet,* ii, 877–897), a syndrome characterised by genu valgum (knock knees) and osteoporosis of long bones is widely prevalent in certain parts of Andhra, and in February 1976 some cases were also being reported from Tamil Nadu and Karnataka states in India. There are three important aspects to the occurrence of this syndrome—it is restricted to regions endemic for fluorosis, the victims are mainly under 25 yr old and there is no mention of this syndrome in the reports by earlier workers who studied fluorosis in those regions of Andhra. The syndrome therefore seems to be a manifestation of fluoride toxicity that has emerged only during the past two decades. But the syndrome does not occur in all fluorotic regions of the states in question, nor in fluorotic regions in many other parts of the Indian subcontinent.

Although the cause of this geographical variation in the manifestation of fluoride toxicity is not yet fully understood, accumulated evidence provides some explanation. The regions where genu valgum is prevalent are mostly fluorotic regions in the vicinity of dams constructed during the past two decades. In Andhra state the syndrome is mainly confined to villages within a radius of 80 km from the Nagarjunasagar dam where there is more than 3.00 p.p.m. fluoride in the drinking water. Villages more than 50 km from the Hopset dam (Karnataka state), mostly with more than 1.00 p.p.m. fluoride in the water, have no cases of genu valgum, but villages within a radius of 5–30 km of this dam, about 50% of which have more than 1 00 p.p.m. fluoride in the water, have a few affected children. The syndrome is also prevalent in villages within 20 km of the Aliyar dam in Tamil Nadu, where the fluoride content in the water is fairly high, the minimum level being 1.9 p.p.m. The prevalence of the syndrome seems to decrease with increasing distance from the dam.

In 1974 the National Institute of Nutrition in Hyderabad suggested that the construction of large water reservoirs near areas endemic for fluorosis may be related to the development of this syndrome. The mechanism involved is not clear. It is known that the construction of large water reservoirs considerably influences the ecology of the surrounding areas by changing the soil conditions for several kilometres from the site of construction. The increased subsoil water subsequently changes the trace element composition of food grains grown on such soil and brings about changes in the trace element composition of water. The level of subsoil water in parts of Andhra where genu valgum is widely prevalent has risen in recent years. An increase in the subsoil water also increases soil alkalinity, producing conditions in which plants are known to pick up more molybdenum from the soil. In this context it is interesting that molybdenum is found in the higher range of concentrations in sorghum and rice grown in fluorotic regions. Sorghum is the staple of the poorer sections of the community and generally contains twice as much molybdenum as does rice. Thus the poorest people, who are most inflicted with the syndrome, may be the most likely to consume a higher content of molybdenum in their diet. Copper and calcium contents of the cereals grown in fluorotic regions have been found to be within the normal range.

Earlier studies (*Br. J. nutr.*, **31,** 351–355; 1974) have shown that a relatively higher dietary intake of molybdenum can cause increased urinary excretion of tissue reserves of copper; the depletion of copper is in turn known to result in poor development of bone matrix and matrix osteoporosis. Because a principal feature of the genu valgum syndrome is osteoporosis of the lower limbs, and because it is found mostly among people whose staple food has a high molybdenum content, the interaction between the two trace elements—copper and molybdenum—seems to be important in the genesis of the syndrome. Krishnamachari (*Indian J. med. Res.*, **64,** 2; 1976) reported the analysis of water samples from 44 fluorotic villages—one sample from each village—of Andhra Pradesh, Tamil Nadu and Karnataka states. He found that "about 70% of villages affected with genu valgum had very low levels of copper in drinking water, the value being mostly below 0.01 p.p.m. None of the villages whose water contained more than 0.1 p.p.m. of copper had genu valgum although their water contained high levels of fluoride".

This association between low copper content in drinking water and the occurrences of genu valgum supports the idea that the relationship between copper and molybdenum in total diet and drinking water could be an important factor in the genesis of the syndrome. ☐

Eukaryotic genome structure and function in Tehran

by Peter Newmark

An IUB–IUPAB International Symposium on Organization and Expression of Eukaryotic Genome was held at the University of Tehran on May 3–6, 1976.

THREE main themes characterised the presentations at the meeting They were the structure of chromatin, the transcription of genes and the organisation of sequences in the eukaryotic genome.

The basic subunit structure of chromatin was the subject of much attention. There is no doubt by now that the string of beads model for chromatin is essentially correct. Each bead or nucleosome comprises eight histone molecules, apparently two each of H2A, H2B, H3 and H4, around which is wound up to 200 base pairs of DNA. Figures given for the exact number of base pairs per monomer nucleosome ranged from 165 in fungi to 210 in chicken erythrocytes These differences are outside experimental error and appear to be characteristic of the source of chromatin. Remarkably, in all cases the nuclease-protected DNA core of the nucleosome remains constant at 140 base pairs. This suggests that all the variability in base pair content per monomer nucleosome lies in the string between the beads which may vary with the tertiary packing induced by H1 and other, as yet unknown, proteins.

Although the way in which the nucleosome DNA is folded around the histones is not clear, the folds must be such as to account for the specific fragments that are generated when chromatin is digested by nucleases. For example H. Zachau (Munich University) presented evidence that the action of DNase II on rat liver chromatin produces 100 base-pair repeat fragments whose generation requires some degree of chromatin superstructure. Since the beads themselves are spaced at roughly 200 base-pair intervals this suggests a specific site of nuclease sensitivity in the middle of each nucleosome.

A very careful kinetic study of staphylococcol nuclease digest fragments of the nucleosome by G. Felsenfeld (National Institutes of Health) revealed that an arithmetic series of fragments of 40, 50, 60 . . . 140 base pairs is first obtained. Further digestion preferentially eliminates the 80 base-pair piece, removes four base pairs from the 130 and 110 pieces and two base pairs from the other pieces. In an extension of these studies Felsenfeld used reconstitution experiments to determine which of the histone molecules are necessary for the generation of the fragments. All the evidence pointed to the primary necessity of the H3 and H4 combination, suggesting that these two histones form the core of the nucleosome. H2A and H2B, however, are also required for the complete generation of the larger fragments that are obtained from chromatin.

The other problem of nucleosome structure is that of the arrangement of the histones. A rewarding approach to that problem has come from crosslinking studies in which chromatin is treated so as to link neighbouring histone molecules. The latest crosslinking agents used by B. J. McCarthy (University of California, Los Angeles) avoid the possible shortcomings of earlier agents that, by their molecular size, required there to be an appreciable space between linked histone molecules. The new agents are tetranitromethane and ultraviolet light. The former crosslinks one region of H2B to H4, the latter links another region of H2B to H2A and sequential treatment of either cells or chromatin with the two agents leads to the formation of an H4–H2B–H2A trimer in very high yield

Suggestive though much of the biochemical evidence might be, most of the participants were reluctant to reveal the model for nucleosome structure that is doubtless lurking in their minds if not in their laboratories. Not so B. Alberts (Princeton) who was happy to reveal the 'Princeton model' in which each nucleosome is formed by the association of a histone tetramer (H2A, H3, H4, H2B) on one of the DNA strands with an identical tetramer on the other strand. The entire nucleosome particle has a dyad axis of symmetry and undergoes an allosteric transition to an open form to allow genetic readout without histone displacement. In spite of its attractive features the 'Princeton model' is still largely speculative and has yet to face a critical experimental test. Its ultimate fate will probably only be determined when it becomes possible to study nucleosome structure by X-ray diffraction. That may not be too far off to judge by a recent Russian success in producing crystals of the 140 base-pair nucleosome core.

Beyond that is the question of the superstructure of the nucleosome string. E M. Bradbury (Portsmouth Polytechnic) described neutron diffraction patterns of chromatin in which the 10.0-nm peak, believed to represent the internucleosome spacing, was not truly meridional. That rules out a linear arrangement for the string in favour of a flat coil that would contain six nucleosomes per turn. A solenoid structure, probably left-handed, was also favoured by F. Crick (MRC Molecular Biology Laboratory, Cambridge) who speculated further that a linear strand of naked DNA might sometimes be found running through the axis of the solenoid.

The problem of understanding transcriptional mechanisms in eukaryotic systems can be approached in a variety of ways. A basic question is whether one can mimic *in vitro* the natural process. Many experiments indicate that with bacterial or eukaryotic polymerases it is possible to achieve some fidelity of transcription as revealed by the detection of specific mRNA sequences. Whereas J. Paul (Beatson

Institute, Glasgow) emphasised the technical difficulties involved in these experiments, R. C. Huang (Johns Hopkins University) described a system in which many of them seem to have been overcome. In particular she described data on the transcription of kappa chain RNA from mouse myeloma cells. Using methods that limited chromatin damage and specifically selecting out only newly synthesised molecules to avoid the complicating factor of endogenous messengers, she demonstrated the faithful transcription of the single-copy kappa chain gene. Furthermore, using cDNA which had been reverse transcribed from the kappa chain messenger, it was possible to show that considerably more of the kappa message was synthesised from the chromatin isolated from a kappa chain-producing cell line than from that isolated from a lambda chain-producing cell line In other words selectivity of transcription can be mimicked *in vitro*. McCarthy presented evidence that generalised that concept In a study of transcription from *Drosophila* chromatin he was able to show that the most abundant sequences present in *Drosophila* heterogenous nuclear RNA are also those that are preferentially represented in the transcript obtained from *Drosophila* chromatin *in vitro*.

What factors control selective transcription from chromatin? According to W. J. Rutter (University of California, San Francisco) at least some of the selectivity resides inherently in RNA polymerase The strongest evidence for that claim comes from his evidence that the transcription of naked yeast DNA by RNA polymerase I in the presence of magnesium ions resulted in a transcript that was specifically enriched in ribosomal RNA sequences. The same may apply to transcription by RNA polymerase III but, in contrast, both yeast RNA polymerase II and *Escherichia coli* polymerase produce largely non-specific transcription on yeast DNA.

Transcriptional selectivity is also determined by chromatin-associated, non-histone proteins. One particular fraction of these proteins, described by A J. MacGillivray (Beatson Institute) seems to contain a putative regulatory protein, but to isolate it at present would be a task equivalent to hunting for a needle in a haystack. E. W. Johns (Chester Beatty) described a different class of proteins known as HMG proteins, some of which are being physically characterised by K. Javaherian (Tehran University). The HMG proteins are histone-related and probably play a structural rather than regulatory role in the chromosome.

As though to demonstrate the lack of understanding of transcription in eukaryotic systems, M. Ptashne (Harvard) was allowed to display the advanced state of the art in bacteriophage λ. For example, his sequencing data explain why a small repressor transcript is associated with the synthesis of small amounts of repressor whereas a larger transcript is associated with increased synthesis. It seems that the two transcripts are produced in approximately equal amounts at different times of infection but the smaller transcript, in contrast to the larger one that is initiated 2,000 base pairs to the right of the coding sequence, does not contain a ribosome binding site and is therefore translated with a relatively low efficiency. Thus this simple virus makes use of both translational and transcriptional controls to modulate synthesis of its repressor protein.

The general organisation of gene sequences in eukaryotes was outlined by E. H. Davidson (California Institute of Technology). Most recently he and W H Klein (California Institute of Technology) have been able to produce evidence in favour of their hypothesis that the 300 base-pair repetitive sequences that are interspersed between the structural genes are involved in the control of expression of the structural genes Only 10–20% of the 5,000 or so families of repetitive gene sequences in the total sea urchin genome were found adjacent to the particular structural genes that are expressed in the gastrula stage. That result indicates that particular repetitive sequences are associated with, and may well therefore control, particular structural genes.

Evidence for a specific example of a controlling region directly adjacent to a structural gene was provided by A. Chovnick (University of Connecticut). He has found a *cis*-acting *Drosophila* mutant at the rosy locus in which there is increased production of xanthine dehydrogenase activity which cannot be accounted for in terms of an alteration in structure, stability or sensitivity of the enzyme The increase in production must therefore be due to the alteration of a controlling region and Chovnick is able to map that mutation within 2–3 kilobases of the well-defined structural gene for xanthine dehydrogenase. He has recently collected new mutants of this type and their mapping is in progress.

The organisation of gene sequences in *Drosophila* is, however, not without its surprises. M. Young (Stanford) describing recent work on the cloning of DNA fragments, reported the occurrence of multiple rather than single copies of two genes coding for abundant mRNA species in *Drosophila* In one case the gene, which codes for a heat shock-induced protein, was found to be locally repeated in multiple tandem blocks separated by spacers. In the other case the gene, probably as a single copy, occurs at 33 different sites scattered throughout the genome. Previous data suggested that such repeats would be unusual and it may therefore turn out that these results are unique for genes making abnormally abundant RNA species.

The most complete evidence for gene organisation in eukaryotes still comes, for technical reasons, from those genes that occur in multiple, tandem copies. An excellent example was provided by M. Birnstiel (Zurich University) who has thoroughly analysed the organisation of the histone genes in the sea urchin. The genes, separated from one another by spacers, are on one DNA strand in the order H4, H2B, H3, H2A, H1 and are transcribed as a single unit starting with the H4 gene.

It is clear that the basic structure of chromatin will shortly be known. That, together with recent technical advances in DNA sequencing and cloning, bodes well for our better understanding of the structure and function of the eukaryotic genome. ☐

Galactic cannon for shooting out radio sources

from John Gribbin

THERE seems to be a clear division in the Universe between the double radio sources associated with many elliptical galaxies and the more spherical outward blast of material from such spirals as Seyferts, even though both kinds of violent event are believed to be produced by explosions in the cores of the respective galaxies. Now, R. H. Sanders has presented calculations of confinement of magnetic field and relativistic particles in a plasma produced in such a way by ram pressure; he shows that just these two kinds of explosion can occur, and that the difference depends on the presence or absence of a massive central source—essentially, a point mass at the galactic nucleus (*Astrophys, J.*, **205**, 335–345; 1976).

The production of paired radio sources, moving outwards on opposite sides of the nucleus of an elliptical galaxy, can also be explained by other models, such as collimated beams of relativistic particles or the fragmentation and ejection of massive objects from the nucleus. Sanders makes no claim that his calculations represent the one true approach, but the success of these numerical simulations is striking, and may encourage some further thought on just how satisfactory the alternative models are. In essence, the problem Sanders has tackled is to find a mechanism for confining the ejected plasma within a narrow cone (vertex angle 12° to 25°); in earlier work with Prendergast (*Astrophys. J.*, **188**, 489–500, 1974) he has already looked at the opposite limitation, when an explosion occurring in the nucleus of a disk of gas (representing a spiral galaxy) can affect gas motions in the plane of the galaxy far from its centre, as opposed to the puzzle for double radio sources of how the emission is confined along the rotational axis.

Within the plane of such a hypothetical disk, expanding plasma produced in a central explosion meets a large mass of material which is pushed along, as Sanders graphically puts it, like a moving snowplough. In the direction of the rotation axis, above and below the plane, the explosion can soon break out into a region of lesser density, and this is obviously of interest to the confinement problem. And, happily in view of the observations, it turns out that the conditions for explosions being able to break out of the plane and for explosions affecting the plane itself over a large distance (essentially depending on the thickness of the disk) are mutually exclusive. But even so, some extra refinement is needed to confine material from explosions which do burst out within the very narrow cones observed in elliptical galaxy radio sources.

Sanders assumed first that there is a constant stellar density core in the nucleus of a giant elliptical, with the gaseous disk structure hypothesised as lying within this core. For a constant thickness disk, an explosion breaking out from the core still expands over almost the entire hemisphere, but this can be overcome by introducing non-uniform conditions, in the form of a massive central point mass. This has two effects. First, the disk is much thinner near the central mass, producing a shape which Sanders likens to a cannon barrel; second, this "barrel" shapes the blast wave from any central explosion, with a degree of focusing just in the required range of 25° and lesser vertex angles.

The implications of this study extend well beyond the "simple" study of radio astronomy, hinting at fundamental differences which may explain the division of galaxies into spirals and ellipticals. There are no Seyferts—indeed, no known spirals—with the double radio source structure typical of radio ellipticals, and on Sanders' evidence the reason may simply be that spirals do not have large point masses at their centres One school of thought holds that elliptical galaxies could have formed in the expanding Universe on the "seeds" of just this kind of central massive object, in the form of a black hole or "retarded core", while spirals formed from collapsing gas clouds in a quite different process (see review in *Nature*, **252**, 445–450; 1974); opponents of this view may of course take the present results as indicating a fatal flaw in the rammed plasma model! But even if we now have a better understanding of the means by which an explosion at the centre of a galaxy can be collimated to produce a double radio source structure, the $64,000 question remains: what is the mechanism of the explosion itself? The energies involved hint very strongly at a gravitational process of some kind, and this again suggests the presence of massive central objects. ☐

Solar–geophysical exchanges discussed in the Soviet Union

from Roger H. Olson

FROM May 17 to 25, a joint US–Soviet conference was held at Kiev University and the Hydrometeorology Service in Moscow to plan for joint research and exchange of data in the fields of solar–geophysical activity prediction, in which the Crimean Astrophysical Observatory is prominent, and the effects of solar–geophysical disturbances on weather and climate. About 30 scientists and specialists attended, representing such subjects as solar physics, geomagnetism and meteorology.

There is much precedent for cooperation in the exchange of solar–geophysical forecasts and data. For years such cooperation has existed because of the need to consider solar activity in radio warning services. The need for cooperation has been emphasised more and more recently because of the need for radiation warnings to space flight operations and because of various other operational problems. In the United States daily forecasts of solar flare and proton events and of geomagnetic activity are available from the National Oceanographic and Atmospheric Administration Space Environment Forecast Center in Boulder, Colorado. In the Soviet Union, such forecasts, particularly of solar activity, are made on demand at several institutions. It was agreed that in the near future daily forecasts would be made from both sides and exchanged in real time, so that each could benefit from the thinking of the other A common system of forecast verification, perhaps based on probability forecasting, will be devised. And efforts will be made to agree on a limited list of indices of solar and geophysical activity, to help avoid confusion.

A system of exchange has been set up between the two countries, under which scientists or technicians from each side can spend several months in the other country, working in more detail on the types of problems discussed at the conference. The first such exchange has already been concluded, with L. Svalgaard of the USA visiting the USSR, and V Loginov visiting the USA The next exchange will be in the spring and summer of 1977, and will involve personnel interested in solar forecasting and data exchange.

The language barrier at the conference was minimised by the presence of expert interpreters and the fact that most of the Russians were conversant with English. But they were not aware of recent results published in English language journals such as *Nature* and *Science*, and the Americans were not familiar with the Russian scientific literature, except that which has been translated into English. ☐

Liposomes to lysosomes

WITH regard to the article "Liposomes to lysosomes" (*News and Views*, **260**, 749; 1976) the statement that "A non-antigenic vesicle would . . prevent the enzyme from eliciting an immunological response . . " is not necessarily correct. Far from preventing an immunological response there are convincing demonstrations in the literature that liposomal entrapment of antigen can augment antibody production. In other words, liposomes can act as immunological adjuvants. This was first described by Allison and Gregoriadis (*Nature*, **252**, 252; 1974) using diphtheria toxoid as the antigen and again by Heath *et al.* (*Biochem. Soc. Trans*, **4**, 129; 1976) using bovine serum albumin. While it may be possible in the future to minimise the response to a given antigen entrapped in liposomes by a suitable choice of lipid composition, charge or size range, the fact that non-antigenic vesicles can increase the immunological response to an antigen remains an incontrovertible fact.

D. CAIRD EDWARDS

articles

Permafrost oxygen isotope ratios and chronology of three cores from Antarctica

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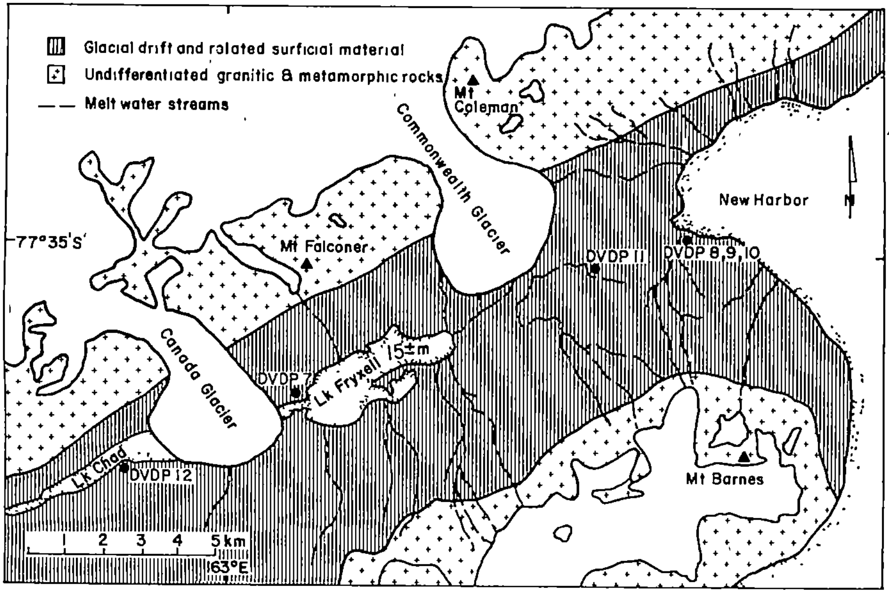
Permafrost core sediments, associated with the last intrusion of the Ross Ice Shelf in the New Harbor region, were deposited in marine (0–85 m deep) as well as freshwater environments (100–125 m). Oxygen isotope ratio measurements on these cores provide palaeoclimatic information and show that the extension of the Ross Ice Shelf predates 150,000 yr BP, *whereas the radiocarbon date of its retreat is about 5,800 yr b.p.*

THE Dry Valley Drilling Project (DVDP) was designed to study the physical, chemical and biological regimen of the subsurface in the arid and ice-free valleys[1] of the McMurdo Sound area of Antarctica. Several sites were cored in Taylor Valley (Fig. 1); this report discusses the oxygen isotope results for cores 8, 9 and 10 drilled near the shore of New Harbor at the mouth of Taylor Valley in the austral summers of 1973–74 and 1974–75. Cores 8 and 9 are from the same site (77°34′37″S, 163° 31′00″E). Core 10 is from nearby at 77°34′43″S, 163°30′ 42″E. The primary core is from hole 8, which extends vertically to a depth of 157.06 m below the surface. Hole 9 was drilled next to hole 8 to a depth of 38.34 m, to obtain a more complete record of an interval of poor recovery of core 8. Hole 10 was drilled in an attempt to penetrate sediments below 157 m; it was terminated at a depth of 206 m when marine groundwater under hydraulic pressure rose up into the hole and froze. The surface elevations of holes 8 and 9 and hole 10 are, respectively, 1.9 and 2.8 m above sea level.

During the past decade there has been a substantial increase in the use of oxygen isotope ratios as a climatic indicator (for instance, the pioneering work on the Camp Century, Greenland ice core[2]). This use is based on the temperature dependence of oxygen isotope fractionation in condensation processes. Although such fractionation follows simple rules for well controlled experimental processes, the interpretation of the ratio changes in nature is more complicated. A change in isotope ratio may be caused, for instance, by either one of the following: (1) change in mean annual temperature, (2) shift in seasonal temperature distribution and precipitation amounts and (3) change in altitude[3]. With a proper evaluation of these factors, a climatic record of the last glacial cycle (~ 100,000 yr) can be obtained for the Arctic and Antarctic ice sheets.

Permafrost cores can extend this record to older glaciations. Of course, the interpretation is more complicated than for ice cores because the accumulation of sediment is not necessarily

Fig. 1 Location map of DVDP drill sites Lower Taylor Valley (after ref. 9).



continuous. In addition, a portion of the ^{18}O record may be missing and replaced by 'younger' water because the temperatures were perhaps not always sufficiently low for permafrost formation, or because post-depositional melting erased part of the record. Our work on permafrost ice gives an unequivocal ^{18}O record of the core waters. The geological and climatic information should, however, be derived mainly in conjunction with other parameters.

The New Harbor holes are close to the shoreline and the upper sediments were deposited in a deltaic environment. Any shift from a marine to a continental environment should be accompanied by a change in oxygen isotope ratios because meteoric waters are, in general, depleted in ^{18}O in comparison to seawater. For example, ice at Byrd Station contains ~ 4% less ^{18}O than undiluted seawater. Brackish waters, composed of a combination of undiluted sea- and freshwater, have intermediate values of ^{18}O deficiency. The ^{18}O ratios of brackish waters depend on the relative portions of undiluted seawater and freshwater and on the ^{18}O content of the freshwater. It is possible to derive the percentage of freshwater dilution and the palaeosalinities from the core isotope data, provided a reasonable estimate of the oxygen isotope content of the freshwater source can be made.

Experimental procedures

The permafrost core sections were kept frozen after collection from the bore hole. They were stored temporarily at McMurdo Station and at present are in the Antarctic Core Library at Florida State University, Tallahassee. Portions were packed in dry ice, shipped to Seattle and stored in a cold room of the Quaternary Research Center until analysed. For analyses, a frozen section of the core with a diameter of 6.2 cm and a thickness of ~ 4 cm was put in vessel A in Fig. 2. The basic purpose of the apparatus shown in Fig. 2 was the complete extraction of meteoric waters from the sediment. After evacuation of vessel A (through traps B and C cooled with a dry ice–acetone mixture), the ice in the sediment was allowed to melt at room temperature and the water was distilled into traps B and C. Evacuation took only several minutes, during which time the sample remained almost entirely frozen. To ensure 100% recovery, we allowed a minimum of 12 h of vacuum distillation. Unfortunately, not only water but also diesel fuel, used as a drilling fluid (DFA), was collected in traps B and C. The resulting emulsion, evidently formed during the early stages of vacuum distillation when transfer was rapid, made it impossible to separate the fuel from the water. Slow condensation in trap D (kept close to 0 °C) yielded water and a floating layer of oil that was removed with the aid of a separatory funnel. The water obtained was equilibrated with CO_2 gas at 25 °C according to standard procedures. The $^{18}O/^{16}O$ ratios of the carbon dioxide were measured with a Nuclide 6–60 mass spectrometer.

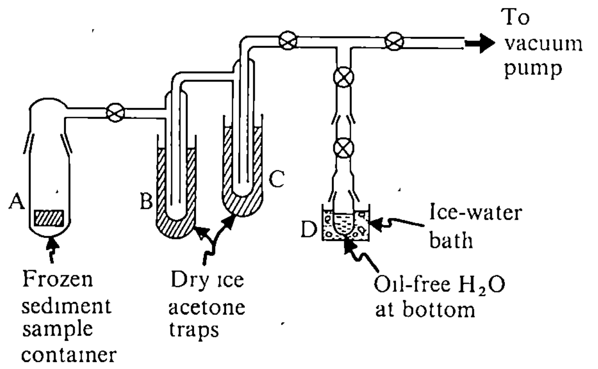


Fig. 2 Apparatus for water extraction (vacuum distillation).

The oxygen isotope ratios are expressed as $\delta^{18}O$ according to

$$\delta^{18}O = \frac{(^{18}O/^{16}O)_S - (^{18}O/^{16}O)_{SMOW}}{(^{18}O/^{16}O)_{SMOW}} \times 1{,}000‰$$

where S denotes sample and SMOW standard mean ocean water. It is clear from the above definition that marine waters as a whole have $\delta^{18}O$ values near 0‰.

^{18}O results and chronology

Oxygen isotope ratio measurements of snow accumulating on the McMurdo Ice Shelf yielded δ values from −32 to −34‰ (ref. 4) and from −28.9 to −31.9‰ (ref. 5). Ice samples collected by us from this ice tongue, however, gave δ values of −1.6 and +2.0‰, because ice within the tongue is derived largely from freezing of seawater and not from snowfall. Surface δ values of Ross Ice Shelf snow at Little America ~ 800 km from New Harbor seem to be near −20‰ (ref. 3). Meltwater from the Lower Wright Glacier, forming the surface water of Lake Vanda in Wright Valley, has a δ value of −29.5‰ (ref. 6). Our own measurements include two samples from Hobbs Glacier, ~ 40 km south-west of New Harbor with δ values of −28.1 and −25.0‰, and one sample from Garwood Glacier, ~ 50 km south of New Harbor with a δ value of −26.6‰. Ice preserved from the last sheet to ground in McMurdo Sound has δ values ranging from −11.4 to −35.3‰, because ice in this sheet originated both from snowfall and freezing of seawater. Lower δ values are encountered for major glaciers fed by high altitude polar plateau ice. Two of our samples from Walcott Glacier (~ 70 km south of New Harbor) gave a δ value of −37.8‰; and a value of −40‰ was given for Taylor Glacier at the head of Taylor Valley (C. H. Hendy, personal communication). From this summary, it seems that current atmospheric precipitation yields $\delta^{18}O$ ratios of ~ −28‰ near New Harbor. Runoff from low-altitude valley glaciers also should have this approximate δ value. Meltwater from Taylor Glacier could, however, introduce lower δ values of ~ −40‰.

It is more difficult to estimate the $\delta^{18}O$ values of freshwater during the coldest interval of the last glaciation. The Byrd Station ice core shows a lowering of ~ 6‰ in δ values[7]. Thus a reasonable guess for $\delta^{18}O$ values during this episode would be −34‰ for local precipitation near New Harbor and −46‰ for nearby polar plateau ice.

The $\delta^{18}O$ values obtained for permafrost waters in the New Harbor cores are plotted in Fig. 3 against core depth. The δ values are all relative to the SMOW standard. The classification in Fig. 3 of sand, gravel, and diamicton units is based on the core descriptions[8,9]. Further studies will undoubtedly provide additional detail, but this is not critical for our discussion.

The oxygen isotope ratios of the permafrost waters give the following picture: (1) Fully marine, or nearly fully marine, conditions down to 85 m; (2) a transition zone to a much less saline environment between 85 and 100 m; (3) freshwater, with possibly some addition of seawater, between 100 and 125 m. The lowest δ values (~ −23‰) are encountered in the sand layer at a depth of 112–114 m. If the freshwater component for this layer was derived solely from local Taylor Valley waters, one would expect δ values of −28 to −34‰. Seawater dilution of respectively 18 and 32% would increase these values to −23‰; (4) a transitional zone from fresh to brackish water ratios between 125 and 130 m; (5) brackish water between 130 and 155 m, with δ values ranging from −7 to −14‰. A mixture of two-thirds seawater and one-third freshwater ($\delta^{18}O$ = −30‰) would yield the average δ value of −10‰; (6) the return to near freshwater values between 150 and 184 m. The lowest δ values, ~ −20‰ are comparable with the ^{18}O values encountered in the 100–125 m interval.

The above calculation of palaeosalinities assumes freshwater contributions of local origin. It is, however, possible that meltwater was derived from Ross Sea ice transported over some distance. As mentioned, higher $\delta^{18}O$ values would result and little or no seawater dilution would be needed to explain the δ values for episodes (3) and (6).

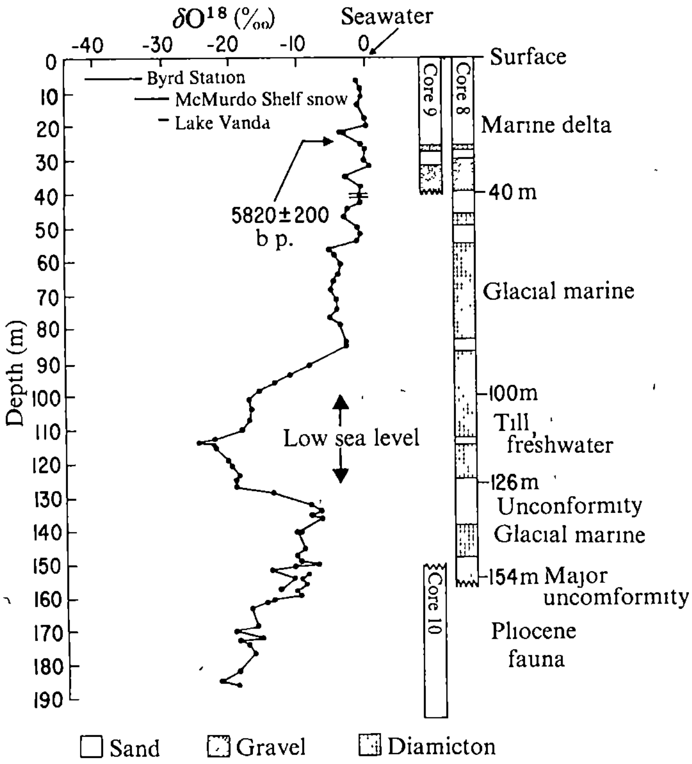


Fig. 3 $\delta^{18}O$ ratios relative to the SMOW standard of permafrost waters in cores 8, 9 and 10 The ^{14}C age at 23 m was obtained for shells in cores 8 and 9.

There is considerable evidence for a succession of grounded Ross Sea ice sheets in the McMurdo Sound region. The most recent grounded ice sheet in McMurdo Sound was not fed by East Antarctic ice dammed behind the Transantarctic Mountains in the dry valley region, nor by local glaciers, but rather by grounded ice that flowed into the sound from an area to the east and south-east that is now covered by the Ross Ice Shelf[10]. The surface contours of the youngest ice sheet occupying McMurdo Sound can be reconstructed from its widespread moraines and drift. Drift associated with the most recent grounded Ross Sea Ice Sheet is also encountered at New Harbor (G. H. Denton *et al.*, in preparation). Thus the upper portion of the core is associated with depositional features of the Ross Sea Ice Sheet and its associated retreat.

Limiting ages for the recession of grounded ice can be obtained from shells collected in silts near the upper marine limit in the McMurdo region[10]. At the mouth of Taylor Valley, New Harbor, raised silts occur up to an elevation of at least 7.5 m. *Adamussium colbecki* shell samples collected at or near the marine limit gave dates of 5,200±70 and 4,950±70 yr b p. (QL-137 and 138; QL=Quaternary Isotope Laboratory at the University of Washington). Likewise, similar shell samples from raised marine deposits at nearby South River gave ^{14}C ages of 5,550±70 (QL-72) and 5,500±60 (QL-96) yr b.p. Moreover, shells preserved in the McMurdo Ice Shelf date back to 5,750±60 yr b.p. (QL-166) These results imply the recession of the youngest Ross Sea Ice Sheet from New Harbor before 5,800 yr b.p.

In calculating all ages of marine shells in this paper a 850-yr correction was applied for ^{14}C ocean water deficiency in McMurdo Sound. This correction was obtained by dating living *A. colbecki* shells collected from near New Harbor in 1974 (850±45 yr b.p.; QL-98). Deeper circumpolar waters have a maximum ^{14}C deficiency of 16%; equivalent with an 'age' of 1,400 yr (ref. 11). Exchange with atmospheric CO_2 will reduce the ^{14}C deficiency in surface waters, and the 850-yr correction seems a reasonable value for this region. This correction is the smallest possible, as nuclear bomb ^{14}C may have increased surface water ^{14}C levels.

Two *A. colbecki* shells in cores 8 and 9, at the 23-m level, gave a ^{14}C age of 5,820±200 yr (QL-191). Evidently the top 20 m of sediment has been deposited since the retreat of the Ross Sea Ice Sheet from the New Harbor region. Moreover, it seems likely that the upper 40–50 m of sands and gravels were deposited in a short interval during, and subsequent to, the recession of the Ross Ice Sheet. This opinion is buttressed by a ^{14}C date of 4,650±70 yr on shells from a bed located 200 m south (QL-161). In our opinion, the top 40 m of sediments have been deposited within the last 10,000 yr. Deposition was in fully marine conditions, as indicated by the ^{18}O data (Fig. 3).

The contacts between diamictons and the stratified water-laid sand and gravel beds are sometimes gradational and do not seem to be erosional. For this reason, the diamictons are considered to be waterlaid[8]. The permafrost waters of the diamicton between 54 and 82 m are nearly fully marine, and the diamicton must have been deposited in seawater through ice rafting.

A fully grounded Ross Sea Ice Sheet possibly could have prevented penetration of seawater to New Harbor, a situation that might produce freshwater ^{18}O ratios in the permafrost. Perhaps this is the reason for the low $\delta^{18}O$ ratios encountered in the diamicton at 87–123 m. On the other hand, it is quite possible that the sea level was lowered sufficiently for emergence of the New Harbor site. Upper marine limits in this area indicate a depression of at least 7.5 m. With a total sea level lowering of ~ 140 m, freshwater δ ratios would be expected somewhere near, or above, the 130 m depth in the core. It is possible that the 125–100 m section with its freshwater permafrost ratios was deposited during such an interval of low sea level.

Few conclusions can be drawn from the ^{18}O record below 125 m. The slight geographical difference in coring sites 10 and 8+9 complicates matters. The overlap in δ values around 154 m (Fig. 3) indicates little difference between the cores, but this may be coincidental.

It is tempting to assign an early–late Wisconsin chronology to the core, with an age of ~ 20,000 yr at a depth of 120 m. Palaeomagnetic work on core 10 seems, however, to indicate an age of several million years for the bottom portion of the core[12]. In addition, foraminifera from DVDP holes 8, 9 and 10 indicate an appreciable older age[13]. These results may be caused partly by the reworking of sediment. Faunal assemblages between 18 and 35 m could perhaps indicate a middle to late Pliocene age range[13], but our ^{14}C age clearly shows this interval to be of Holocene age. In this instance, older Ross Sea sediment has been transported to the New Harbor site through the action of the Ross Ice Sheet. This leaves the possibility of more core sections with reworked materials. A solution to this problem may be coming from additional palaeomagnetic determinations.

Potassium–argon dates on basalt flows that occur in the southern area of McMurdo Sound and that predate the last extension of the Ross Sea Ice Sheet show that the last ice sheet growth postdates 150,000 yr (G. H. Denton *et al.*, in preparation). The fabric and lithology studies of S. C. Porter and J. Beget in the Quaternary Research Center suggest that the thick diamicton near the 100-m depth probably represents grounded glacier ice of Ross Sea origin whereas other diamictons in the core probably are of glacial marine origin. Again one would infer a short chronology if this episode was tied to the last expansion of the Ross Sea Ice Sheet. In our opinion, sediment deposition above the 126-m depth is restricted to the last 100,000 yr. A major unconformity is suspected near 154 m.

Foraminiferal composition below this depth indicates a Pliocene age[13].

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Quarks in the early Universe

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The quark theory of hadrons provides a valuable tool for interpreting conditions during early epochs of the Universe. In particular, an asymptotically free theory of quarks seems to rule out the possibility that the present-day entropy of the Universe could be due to dissipation of low level fluctuations. Apparently either the early Universe was inhomogeneous on small mass scales or anisotropic.

It is by now almost a certainty that hadrons consist of quarks bound by vector gluons[1]. The fact that free isolated quarks have never been observed evidently means that in ordinary conditions quarks are permanently bound inside hadrons. There are, in fact, reasons[2] for believing that the force between quarks increases with distance rather than falling off as electromagnetic forces do. On the other hand, high energy electron–proton scattering experiments suggest[1] that when the separation between quarks is $< 10^{-13}$ cm, then the force between the quarks is rather weak. Thus one might guess that when the density of matter is increased to the point where the mean distance between quarks in different hadrons is $\ll 10^{-13}$ cm, then the hadrons will disappear and matter can be described as quark 'soup'[3].

Equations of state

If one accepts the idea that matter consists of free quarks above a certain density then it becomes possible to work out the properties of matter at superhigh densities. For example, the energy density of matter at superhigh densities will have the form

$$\rho = An^{4/3} + B \qquad (1)$$

where n is the baryon number density and A and B are constants. The constant A depends on the quark–gluon coupling constant g and entropy per baryon; at zero entropy[4]

$$A \simeq 9/4(3\pi^2/\kappa)^{1/3}[1 + (8g^2/3\pi)]\hbar c$$

where κ is the number of quark 'flavours' contributing to the energy density. The constant B is a universal energy density associated with quarks[5]; the observed properties of hadrons suggest that B is $\sim 10^{35}$ erg cm^{-3}. From equation (1) one finds that the pressure of matter at superhigh densities is

$$P = \frac{A}{3}n^{4/3} - B \qquad (2)$$

That the pressure given by equation (2) is negative when the baryon number density is $< (3B/A)^{3/4}$ shows[4] that below a certain pressure quark matter will fragment into hadrons. From equations (1) and (2) one finds that $P \to \rho/3$ when $\rho \gg 4B$. Thus the speed of sound in matter $(\mathrm{d}P/\mathrm{d}\rho)^{1/2}$ asymptotically approaches $c/\sqrt{3}$ at superhigh densities.

That matter at superhigh densities can be described as a gas of quasi-free quarks is of course a highly useful piece of information when one wishes to discuss those astrophysical situations where superdense matter occurs. For example, knowing the behaviour of matter at superhigh densities allows extrapolation of the behaviour of an expanding universe back to very early times. In the 'big bang' model[6] of the Universe, energy densities $\gg 4B$ will occur at times $t < 10^{-5}$ s, and thus the free quark picture should be valid at those times. In the following I will show that this has important implications for the question of the origin of the entropy of the Universe and whether primordial black holes exist.

The entropy of the Universe

The good agreement between the observed abundance of helium and the abundance of helium theoretically expected[7] from hot big bang nucleosynthesis suggests that the Universe was already hot enough to be radiation dominated when the Universe was 1 s old Further, the agreement between calculations[8] of the amounts of deuterium, ^{3}He, and ^{7}Li formed during the time of helium synthesis and the observed abundances of these isotopes is strong evidence that the entropy of the Universe at 1 s was approximately equal to its present value, which is essentially just that contained in the cosmic microwave background and is, in Boltzmann's constant units, $\sim 10^9$ per baryon. Why the Universe has such a large entropy per baryon is unclear.

Ya. B. Zel'dovich has suggested[9] that the entropy results from the dissipation of sound waves during very early epochs. These sound waves could be the result of small scale primordial density fluctuations. The existence of primordial density fluctuations on very large mass scales may explain the origin of galaxies. Zel'dovich points out that if there were the same level of fluctuations that gave rise to galaxies present on very small mass scales, then the observed entropy per baryon can be explained provided the equation of state of matter at superhigh densities approaches the form $P = \rho c^2$. The assumption that the pressure approaches ρc^2 at superhigh densities is crucial because it leads to very large internal energies per baryon. One can show that if $P = \rho c^2$ then the energy per baryon $\propto n$. Although such an asymptotic behaviour is consistent with phenomenological nucleon–nucleon potentials[10], it is not consistent with the free quark picture.

Asymptotic freedom and entropy

The energy density associated with a sound wave is

$$u_s = \varepsilon^2 \rho c_s^2 \qquad (3)$$

where $\varepsilon \equiv \delta\rho/\rho$ measures the maximum deviation from uniform density and c_s is the speed of sound. If the acoustic energy density u_s is completely dissipated then an equivalent amount of heat energy will appear. Some of this heat energy will appear in the form of radiation (gluons and photons), and if this radiation is in thermal equilibrium then the entropy per baryon S will be

$$S \simeq \rho_r^{3/4}/n \qquad (4)$$

where ρ_r is the radiation energy density. If $\rho = (n/M)^2$ then one finds from equations (3) and (4) that the radiation entropy per baryon will be large when $n \gg (M/\varepsilon)^3$. If $M \sim 1$ GeV this corresponds to densities $\gtrsim \varepsilon^{-3} \times 10^{17}$ g cm^{-3}. On the other hand, if asymptotic freedom (equation (2)) applies at superhigh densities then one finds that the radiation entropy per baryon resulting from dissipation of a sound wave can never be larger than $(\varepsilon/3)^{3/2}$. This quantity can never be large in an approximately homogeneous Universe where $\varepsilon \ll 1$.

Even though the entropy per baryon resulting from dissipation of a single sound wave is small, one might still ask whether dissipation of sound waves over cosmological time scales can lead to an appreciable buildup of entropy. Suppose, for example, that the dissipation of acoustic energy were fairly efficient, so that the energy density u_s at time t is dissipated in a time t. Then the radiation energy density ρ_r will evolve with time according to

$$\frac{d\rho_r}{dt} \simeq \varepsilon^2 \frac{\rho_m}{t} - 2\frac{\rho_r}{t} \qquad (5)$$

where ρ_m is the density of matter. The second term on the right hand side of this equation represents the effect of the expansion of the Universe on the radiation energy density. If we assume $\rho_r = 0$ at some initial time t_0, then equation (5) together with the equations of general relativity imply that

$$\frac{\rho_r}{\rho_m} = \left[\frac{t}{t_0}\right]^{\varepsilon^2} - 1 \qquad (6)$$

We see that $\rho_r \gg \rho_m$ only when $\ln(t/t_0)$ is large compared to ε^{-2}. It is not entirely clear what one should choose for t_0, but the quantum theory of gravity seems to imply[11] that t_0 should be identified with the Planck time, $t_p \equiv (\hbar G/c^5)^{1/2} \simeq 10^{-43}$ s. If this is the case an appreciable entropy could not have been generated by the time $t = 1$ s unless $\varepsilon \gtrsim 0.4$.

As to whether $\varepsilon > 0.4$ will in fact lead to a large entropy per baryon one must ask whether it is possible to have efficient dissipation over long periods of time. Actually, if ε is close to unity there will be some dissipation just from the fact that in such a situation black holes would be prolifically formed[12], and these black holes emit thermal radiation[13]. This radiation causes small black holes of mass M g to disappear completely in a time $\sim 10^{-27} M^3$ s (ref. 14). If we assume that black holes formed at time t have masses typically of the order of the Hubble mass $M_H = (4\pi/3)\,\rho\,(ct)^3$, then production of a large entropy per baryon by the time $t = 1$ s would require prolific production of black holes at times earlier than 10^{-28} s. It can be shown that the entropy per baryon generated by the decay of a black hole formed at time t is $\simeq (t/t_p)^{1/2}$. Thus at least 99% of the baryons initially present would have to be processed through black holes to produce the observed entropy of $\sim 10^9$ per baryon.

An anisotropic Universe?

By choosing a value for ε close to unity for times $t < 10^{-28}$ s one can apparently produce a very large entropy per baryon by the time the Universe is 1 s old. The main difficulty with this explanation is that in order not to conflict with the observed large scale homogeneity of the Universe and lack of a large present-day density of primordial black holes[15], the value of ε must somehow decrease from being close to unity for very small mass scales to being rather small ($\lesssim 0.01$) on very large mass scales. We do not understand how or why ε should vary with mass scale. One possible way out of this difficulty might be to suppose that the Universe is actually rather homogeneous on all mass scales but in addition is anisotropic[16]. It has been shown[17] that if weakly interacting particles are present in such a Universe then the pattern of anistropic expansion can be altered. In particular, if the mean free time between collisions is comparable with the expansion time, then strong damping of the anistropy can occur[18]. The mean time for a large angle quark–quark scattering is

$$\tau \simeq \frac{1}{\pi g^2}\,\frac{E_q^2}{n_q c} \qquad (7)$$

where E_q is the quark energy and n_q is the quark density. Since $E_q \propto V^{-1/3}$ in a collision dominated Universe we have $\tau \propto V^{1/3}$ as long as $\tau \ll t$. The expansion time is, however, proportional to V in an anisotropic Universe. Thus one will always have an initial stage of the Universe where τ is longer than the expansion time. During this initial period when the quarks are completely free, the quarks moving in certain spatial directions will be 'blueshifted' in energy rather than redshifted as in a collision-dominated Universe, and one will build up counter-streaming beams of energetic quarks along certain spatial directions. Because these beams are unstable from the quark plasma analogue of the two-stream instability, the buildup of these beams will ultimately result in entropy generation. One can calculate the rate of entropy production from the theory of two-stream instabilities in relativistic plasmas; however, this mechanism of entropy production lacks appeal at the present time as an explanation of the observed entropy because the duration of the anisotropic period and hence the entropy produced is a free parameter that can only be determined from the observed value of the entropy.

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Structural patterns in globular proteins

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A simple diagrammatic representation has been used to show the arrangement of α helices and β sheets in 31 globular proteins, which are classified into four clearly separated classes. The observed arrangements are significantly non-random in that pieces of secondary structure adjacent in sequence along the polypeptide chain are also often in contact in three dimensions.

One of the central problems in molecular biology is the formation of the native structure of a protein from the newly synthesised unfolded "structureless" polypeptide chain. In suitable conditions this process occurs spontaneously and the final conformation is determined solely by the amino acid sequence. As the random search of all possible conformations of the whole molecule would take an impossibly long time, this process probably involves intermediate structures that allow the protein to find its native conformation rapidly. Considerable experimental and theoretical effort has been devoted to trying to establish the nature of these intermediates.

In this article we first use a simple two-dimensional representation to illustrate the known conformations of 31 proteins. After classifying these known protein structures into four classes, we show that there is a strong tendency for pieces of secondary structure that are close together along the sequence also to be in close contact in the final three-dimensional structure. Such locally ordered regions, which are referred to here as folding units, associate to form the whole protein molecule, or in the case of some of the larger proteins, to form domains.

Diagrammatic representation of protein structure

Protein conformations are very complicated: it is not easy to comprehend the three-dimensional structure of a single protein, let alone compare many such structures. We have chosen to use a schematic two-dimensional representation similar to that used by certain other workers[1,2], and referred to here as topology/packing diagrams. The following rules were used in preparing our topology/packing diagrams for each protein. First, the α-helical and β-sheet chain segments are identified. As a β sheet can be formed from pieces of chain that are distant along the sequence, we use 'β' strand to refer to a single piece of chain that forms one strand of a β sheet. Second, a viewing direction is defined so that most segments of secondary structure are viewed end on. Third, the protein is rotated about the line of sight until the β strands lie in a horizontal plane (the twist of the β sheet is removed by flattening the sheet), and the front end of each β strand is drawn as a rectangle in the diagram. (This step is omitted if there is no β sheet.) Fourth, a circle representing the front end of each α helix is drawn, taking care to put segments that are close in space close together in the diagram. Finally, the segments are connected by bold or thin arrows (from the N to C terminal) that indicate whether the connection is at the near or far end, respectively, of the α helix or β strand. The scale of the real protein is preserved by making the separation of interacting α helices 10 Å, of hydrogen-bonded β strands 5 Å, and of other interacting β strands 10 Å. The diameter of the α-helix circle is 5 Å, and the β-strand rectangle is 4 × 5 Å.

Not all known protein conformations are included in the topology/packing diagrams given here (Figs 1–4). In some cases a particular structure is omitted as it is closely related to a protein that is shown: we show only one example of the immunoglobulin family[8–11], the trypsin family[41,42] and the haemoglobin family[43,44]. For a few proteins we could not find sufficiently clear pictures in the literature to be able to produce the diagrams (high potential iron protein[45], cytochrome c_2 (ref. 46), the catalytic part of alcohol dehydrogenase[30], malate dehydrogenase[47], ferredoxin[48], rhodenase[49], carbonic anhydrase[50] and soya bean trypsin inhibitor[51]). As these omitted proteins seem quite normal and fall into the present classification their omission should not affect our study.

Four clearly defined classes

The topology/packing diagrams of the 31 proteins are arranged into four classes defined as follows: (I) all-α proteins have only α-helix secondary structure (Fig. 1); (II) all-β proteins have mainly β-sheet secondary structure (Fig. 2); (III) α+β proteins have α-helix and β-strand secondary structure segments that do not mix but tend to segregate along the polypeptide chain (Fig. 3), and (IV) α/β proteins have mixed or approximately alternating segments of α-helical and β-strand secondary structure (Fig. 4).

Class I proteins are built up from α helices: more than 60% of the residues adopt the helical conformation. Because strongly interacting α helices are not always parallel (especially if the helices are short), the topology/packing diagrams do not show the packing of helices very accurately (Fig. 1). Of the three α-helical proteins shown, myohaemerythrin is most accurately represented as all the helices are parallel to the line of sight. The same packing of almost parallel α helices has

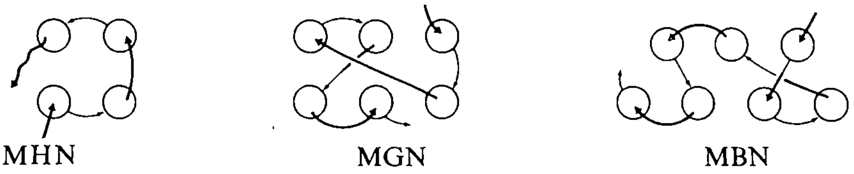


Fig. 1 Topology/packing diagrams of the following three all-α proteins (three-letter abbreviations have been assigned to all the proteins presented here): myohaemerythrin (MHN)[3]; myogen (MGN)[4], and myoglobin (MBN)[5,6] As the axes of adjacent α helices are not always parallel and are sometimes at right angles, the diagrams cannot show the three-dimensional arrangement of helices very well.

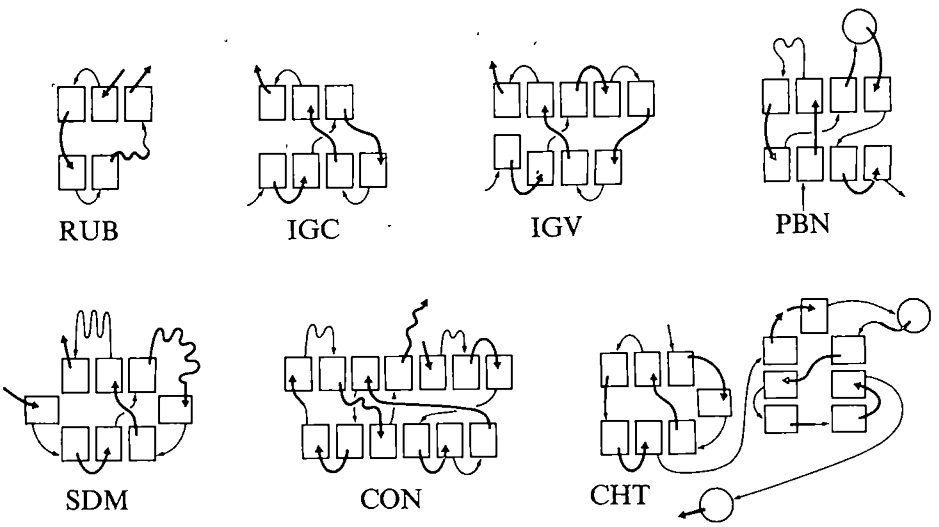


Fig. 2 Topology/packing diagrams of the following seven all-β proteins in order of increasing size: rubredoxin (RUB)[7]; immunoglobulin constant region (IGC)[8,9], immunoglobulin variable region (IGV)[10,11]; prealbumin (PBN)[12]; superoxide dismutase (SDM)[13]; concanavalin A (CON)[14,15], and chymotrypsin (CHT)[16,17] If the rectangles representing two β strands are very close together then the strands are hydrogen-bonded together In some cases the associations are less clear in the diagrams: the final β strand of IGV also hydrogen bonds to the first strand; the first strand of SDM hydrogen bonds to both the second and final strands, and the fifth strand hydrogen bonds to both the fourth and sixth strands; in each domain of CHT, the second strand hydrogen bonds to both the first and third strands. The approximate twofold symmetry relationship between the upper and lower β sheets of the immunoglobulin regions and superoxide dismutase is clear (the twofold axis lies horizontally between the sheets).

been found in low resolution X-ray studies of tobacco mosaic virus coat protein[52,53] and electron microscopy of purple membrane protein[54].

Class II proteins (Fig. 2) are built from β sheets stacked to form a layered structure. Although the number of β strands in each sheet varies from two (in rubredoxin) to seven (in concanavalin A), there are always two layers of β sheet. Adjacent β strands in these proteins run in opposite directions to form antiparallel sheets. All these class II proteins, except chymotrypsin, have only one domain, and in chymotrypsin the two domains have identical topological connections. Often the strands that occur near the ends of the polypeptide chain are positioned in the middle of the β sheet so that they are well stabilised with hydrogen-bond associations to two neighbouring β strands. In three cases, the β strand at the edge of one β sheet also hydrogen bonds to the other β sheet closing one edge of the double layer (in the immunoglobulin variable region, superoxide dismutase and chymotrypsin), and in one of these (superoxide dismutase) both ends of the double layer are closed to form a barrel of β strands. The close similarity of the chain fold in the immunoglobulin variable region and superoxide dismutase, which has been pointed out before, is clear in Fig. 2.

Class III, the α+β proteins (Fig. 3), consist of a mixture of all-α and all-β regions within the same polypeptide chain. Often there is a cluster of helices at one or both ends of the β sheet, which is almost always built up from antiparallel strands. Only the three largest α+β proteins can be split into two relatively stable domains, and in each case one domain is mainly α helical while the other is mainly β sheet (T4 lysozyme, papain and thermolysin).

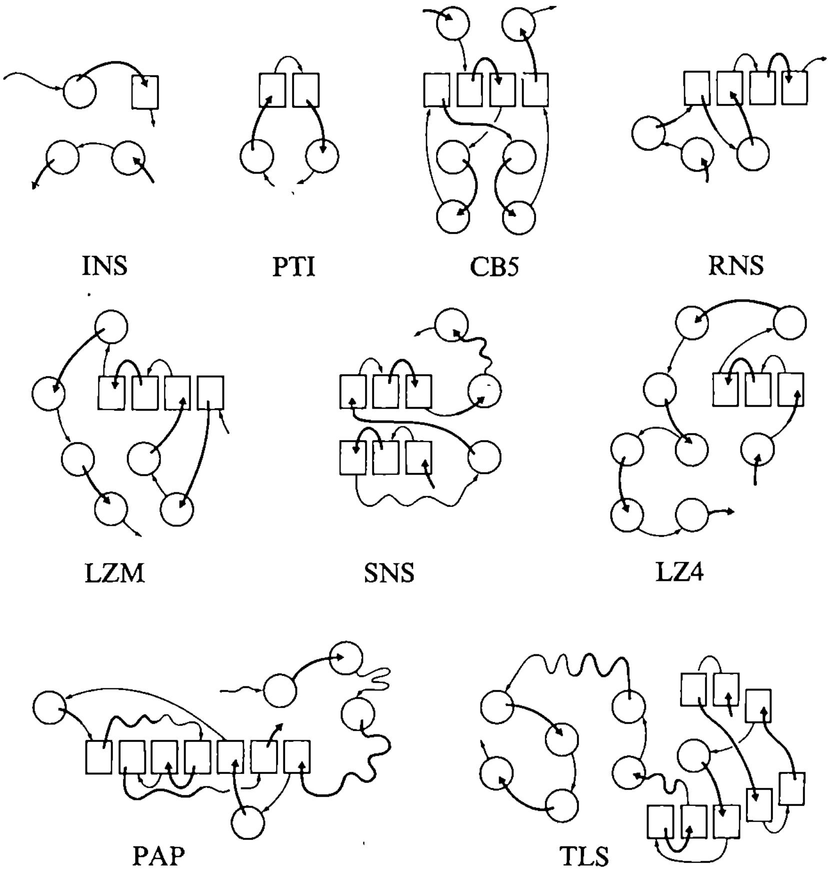


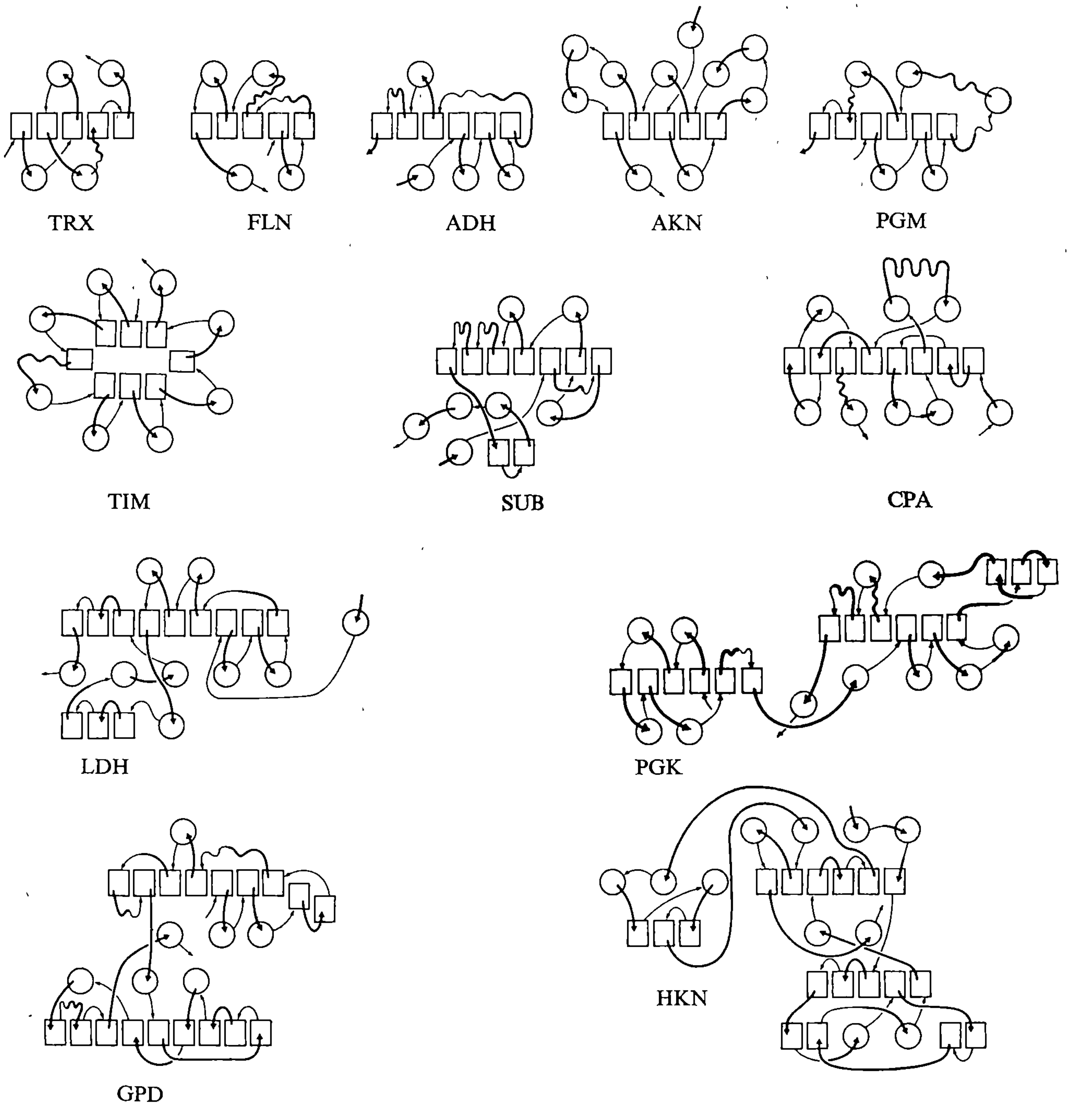
Fig. 3 Topology/packing diagrams for ten α+β proteins in order of increasing size: insulin (INS)[18]; pancreatic trypsin inhibitor (PTI)[19]; cytochrome b_5 (CB5)[20]; ribonuclease (RNS)[21]; hen lysozyme (LZM)[22]; staphyloccocal nuclease (SNS)[23]; T4 lysozyme (LZ4)[24]; papain (PAP)[25], and thermolysin (TLS)[26] The diagrams show that the two lysozyme structures are more similar than thought previously[24]; analogy with the mammalian enzyme supports the idea[24] that the active residues of T4 lysozyme must be a glutamic acid at the end of the α helix preceding the β sheet and an aspartic acid at a bend in the β sheet (Glu 11 and Asp 20, respectively)

Class IV proteins, the α/β proteins (Fig. 4), have α helices and β strands that occur one after the other so that most α helices are separated by β strands along the sequence and vice versa. Most of these proteins have a single sheet surrounded by α helices, but in some of the larger proteins there are extra, smaller β sheets. The α helices pack on both sides of the β sheet, with α helices that follow one another along the chain, often on the same side of the β sheet. The main β sheet of each protein has between five and nine strands and consists mainly of parallel strands. The extra β sheets are smaller, often antiparallel, and more like the β sheets of the α+β proteins in class III (SUB and LDH in Fig. 4). Only the five largest of these α/β proteins (more than 300 residues) can be separated clearly into two or three domains. A common feature of these proteins is the order of the β strands in the β sheet: in most cases the first strand is in the middle of the sheet, with the following strands along the chain added first to the right and then to the left of the first strand. Because most of the α/β proteins shown here are enzymes in the glycolytic pathway and have to bind a common coenyzyme, NAD, some of the common features in Fig. 4 may be a result of these proteins having evolved from a common ancestor[2].

General features of known proteins

The interior hydrophobic core so characteristic of globular proteins is generally formed by contacts between and among α helices and β strands. The pieces of chain (often quite short) that connect these regions of secondary structure are generally

Fig. 4 Topology/packing diagrams of 12 α/β proteins in order of increasing size: thioredoxin (TRX)[27]; flavodoxin (FLN)[28,29]; alcohol dehydrogenase coenzyme domain (ADH)[30]; adenyl kinase (AKN)[32]; phosphoglycerate mutase (PGM)[31]; triose phosphate isomerase (TIM)[33]; subtilisin (SUB)[34]; carboxypeptidase (CPA)[35]; lactate dehydrogenase (LDH)[36]; phosphoglycerate kinase (PGK)[37,38]; D-glyceraldehyde-3-phosphate dehydrogenase (GPD)[39]; and hexokinase (HKN)[40]. Because the upper and lower domains of GPD and HKN are not directly under the upper domains, the interactions are not as strong as implied by the figures The arrangement of the lower domain of HKN must be regarded as only tentative as it was determined from a mono drawing of this very large protein[40].



exposed to solvent and contain most of the hydrophilic and bend-promoting residues. Most proteins can be considered crudely as a layered sandwich structure, with each layer consisting entirely of either α helices or a β sheet. In the all-α proteins or all-β proteins, there are only two such layers. In the α+β proteins there are also often two layers, and often one layer consists of α helices and the other is a β sheet, though more mixed arrangements occur. In the α/β proteins, there are three layers, with a layer of α helices on each side of the central β sheet. Triose phosphate isomerase may seem an exception in that there are four layers with a central β-sheet sandwich surrounded on both sides by α helices, but this protein can also be considered as a layer of β sheet and a layer of α helices rolled into a cylinder so that every β strand hydrogen bonds to two others. Although β strands are sometimes surrounded on all sides by other segments of secondary structure, α helices are almost always only partially buried. It is also rare to find an α helix that is not in contact with at least one other α helix.

Very often several secondary structure segments that are adjacent along the polypeptide chain also interact strongly in three dimensions to form what is defined here as a 'folding unit'. There are 12 possible combinations of α helices and β strands into sequences of two or three adjacent segments of secondary structure: (αα), (αβ), (βα), (ββ), (ααα), (ααβ), (αβα), (βαα), (αββ), (βαβ), (ββα) and (βββ) Not all these secondary structure segments interact to form a stable complex. Those combinations containing a single β strand must be excluded as that strand can neither interact strongly with an α helix nor hydrogen bond to another β strand in the folding unit to form a β sheet. The most important folding units, therefore, consist of the following groups of adjacent secondary structure segments: (αα), (ββ), (βββ) and (βαβ). The (αα) folding unit consists of a pair of α helices adjacent in sequence that are arranged with their axes approximately antiparallel and are in van der Waals' contact (Fig. 5*a*). The (ββ) folding unit consists of two β strands that fold back and hydrogen bond together into a β sheet with two antiparallel strands (Fig. 5*b*). The (βββ) folding unit is just an extension of the (ββ) folding unit with an extra β strand forming a β sheet with three antiparallel strands arranged in a simple zigzag. The (βαβ) folding unit consists of a β sheet with two parallel strands in van der Waals' contact with an α helix antiparallel to these strands (Fig. 5*c*). Three other possible groups, (ααα), (αββ) and (ββα), are rare in globular proteins, difficult to identify unambiguously and can be considered as formed from (αα) or (ββ) folding units.

Table 1 gives the number of times the different folding units occur in all the globular proteins considered here. For some of the α/β proteins (class IV) adjacent (βαβ) folding units have a β strand in common. As folding units are defined here as independent subassemblies of secondary structure, a particular segment of secondary structure should not be part of two folding units. A new type of folding unit, referred to as (βαβ)′, was introduced to take care of the case (βαβαβ), that is, where a (βαβ) folding unit shares a β strand with an adjacent (βαβ) unit. For example, triose phosphate isomerase is formed from eight (βαβ)′, folding units.

In the three all-α proteins there are eight (αα) folding units. If every α helix in these three proteins was in contact with the adjacent helices along the sequence, the maximum possible number of (αα) folding units would also be eight. In the seven all-β proteins there are 20 (ββ) and four (βββ) folding units, respectively; the maximum numbers that could be formed are 24 (ββ) and six (βββ). In the nine α+β proteins, there are 12 (αα), five (ββ) and six (βββ) folding units, respectively; the maximum numbers that could occur for these proteins are 16 (αα), seven (ββ) and six (βββ). In the twelve α/β proteins there are six (αα), 10 (ββ), four (βββ), 18 (βαβ) and 12 (βαβ)′, folding units, respectively; the maximum possible numbers are 12 (αα), 12 (ββ), four (βββ), 23 (βαβ) and 15 (βαβ)′.

The above results show that for each class of protein the actual number of folding units observed is close to the maximum possible number. For all the 31 proteins together there are 26 (αα), 35 (ββ), 14 (βββ), 18 (βαβ) and 12 (βαβ)′ folding units, respectively. A total of 242 segments of secondary structure belong to one or other of these folding units out of a total of 361 segments (67%). It is interesting that in the all-β proteins more β strands are in (ββ) rather than (βββ) folding units, whereas in the α+β proteins more β strands occur in (βββ) rather than (ββ), folding units.

Rao and Rossmann[55] concluded from an examination of four proteins that a structure consisting of three parallel β strands and two joining α helices was a common structural building block in proteins. (This structure consists of two overlapping (βαβ)′ folding units.) They also noticed that the structure had a unique hand, in that the polypeptide chain is directed from β to α to β in a clockwise sense (Figs 4 and 5*c*). Sternberg and Thornton[56] have confirmed this handedness from a study of many protein structures and concluded that it arises from the twist of the β sheet. In another analysis of β sheets in protein structures, Richardson *et al.*[57] also noticed the high frequency of (ββ) and (βαβ) folding units.

Statistical significance of patterns

Another way to assess the statistical significance of the arrangement of secondary structure in known proteins is to estimate the probability that a particular observed pattern would occur by chance. As the topology/packing diagrams represent the arrangement in space of α helices less well than of β strands, we first considered the statistical significance of the connectivity of the strands in the β sheets of proteins in classes II to IV. A computer program was used to generate random permutations of the order of the different β strands along the sequence while preserving the relative positions of the strands in the β sheets of the particular protein (the direction of the chain in the segments was not taken into account). For each of the 1,000 permutations generated for a particular protein, we counted the number of times a pair of β strands adjacent in the permuted sequence were also in contact in the β sheet (referred to as the number of adjacent contacts). This same

Fig. 5 The folding of the polypeptide chain in the three commonly occurring folding units: (αα), (ββ) and (βαβ) The ribbon illustrates the path of the backbone with the arrows directed from the N to C terminals. Below each drawing of the chain fold is shown the representation of the particular folding unit used in the topology/packing diagrams of Figs 1–4.

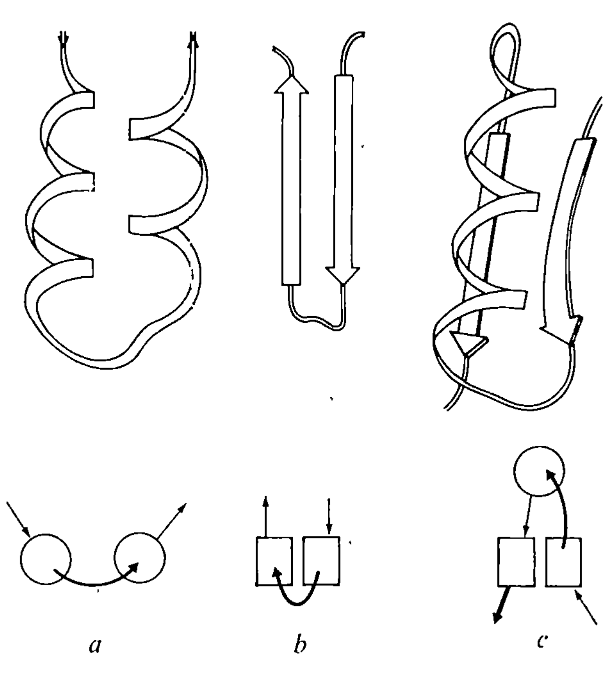


Table 1 Folding unit counts, β-sheet patterns, β-strand polarity and chance probability of 31 proteins

Class and protein	No. of folding units					β-Sheet pattern†	Strand polarity*		Probability
	(αα)	(ββ)	(βββ)	(βα)	(βαβ)′		*N*p	*N*ap	
All-α									
Myohaemerythrin	2								0.33
Myogen	3								0.42
Myoglobin	3								0.14
All-β									
Rubredoxin		2				−+− +−		3	0.37
Ig constant		3				−+− −+−+		5	0.14
Ig variable		3	1			−+−+− −+−+		7	0.006
Prealbumin		2				−+−+ +−−+	1	5	0.16
Superoxide dismutase		3				−+− + + −+−		8	0.007
Concanavalin A		2	2			+−+−+−+ +−+−+−		11	0.001
Chymotrypsin		5	1			−+− + + − − −+− + + − −		12	0.05
α+β									
Insulin	1								1.0
Trypsin inhibitor		1				+−		1	1.0
Cytochrome b5	2	1				−−+−	1	2	0.93
Ribonuclease	1		1			−+−+		3	0.08
Lysozyme (hen)	2		1			+−+−		3	0.51
Staph.nuclease			2			+−+ +−+		4	0.006
Lysozyme (T4)	3		1			+−+		2	0.33
Papain	1		1			−−+−+−+	1	5	0.54
Thermolysin	3	3				−++ −+++−	3	3	0.17
α/β									
Thioredoxin		1		1		−−−+−	2	2	0.56
Flavodoxin				2	1	−−−−−	4		0.15
Alcohol dehydrog.				2	1	−−−−−−	5		0.21
Adenylade kinase	2					−−−−−	4		0.56
Phosphoglycerate mutase		1		1		−+−−−−	3	2	0.59
Triose phosphate isomerase					8	−−− − − −−−	8		0.001
Subtilisin	1	1		2		−−−−−−− +−	6	1	0.24
Carboxypeptidase		1		1		++−−−−−−+	4	3	0.25
Lactate dehydrog.	1		2	2	2	−+−−−−−−− −+−	6	4	<0.001
Phosphoglycerate kinase	1			4		+−+ −−−−−−− −−−−−−+	9	3	<0.001
Glyceraldehyde phosphate dehydrog		3		3		−−−−−−−−−+ ++−+−++−+	9	7	<0.001
Hexokinase	1	2	2	1		+− ++ −−−+− +−+ +−+−−−	5	8	0.003

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*The numbers of parallel β-strand associations in the β sheet(s) are given in the column headed *N*p, and the number of antiparallel associations are given in the column headed *N*ap.

†The β-sheet pattern is taken from Figs 2–4 with a plus sign to mark β strand that runs away from the viewer (into the paper), and a minus sign to mark a β strand that runs towards the viewer (out of the paper).

number was obtained for the native protein arrangement from Figs 1–4. As the α helices are ignored, β strands in contact in the sheet but separated in sequence by only one α helix were also counted as adjacent contacts. The statistical significance of the native arrangement was taken as the probability that a randomly generated permutation of strand order in the particular β sheet would have at least as many adjacent contacts as the native arrangement. These probabilities (P) are given in the last column of Table 1 and vary from 1.0 to 0.001 with a geometric mean of 0.05. For the smallest β sheet consisting of only two β strands, any permutation of the strands will leave them in contact in the sheet so the probability of having at least one adjacent contact is 1.0 (for example, trypsin inhibitor, Fig. 3) For a β sheet with three strands, there is a one-third chance of forming an antiparallel arrangement with two adjacent contacts (for example, T4 lysozyme, Fig. 3). As the size of the β sheet increases the chances of having many such adjacent contacts decreases. For example, the β sheet in both cytochrome b_5 and ribonuclease consists of four strands (Fig. 3), but the arrangement in the former protein is almost random (one adjacent contact, $P = 0.33$), whereas that in the latter is highly significant (three adjacent contacts, $P = 0.08$). One of the three α/β proteins with a five-stranded β sheet (Fig. 4), flavodoxin has a more significant pattern (three adjacent contacts, $P = 0.15$) than the other two (both thioredoxin and adenylate kinase have two adjacent contacts each and $P = 0.56$). The patterns found in the proteins with the biggest β sheets are very significant, with P values less than 0.01, and in the case of some of the dehydrogenases, less than 0.001. These results show that β strands that are adjacent in sequence are in van der Waals' or hydrogen-bonding contact much more often than expected by chance. Had the connectivity of the α helices also been considered, the observed patterns would be even more significant as then there would be many more segments of secondary structure to permute. The significance of the patterns of the all-α proteins was also estimated in this way but now the helices could be in contact both vertically and horizontally in the topology/packing diagrams. The results of this calculation (Table 1) show that none of these patterns of the all-α proteins is statistically very significant.

Parallel and antiparallel β sheets

Table 1 also shows the polarity of the β strands in the β sheets of the proteins considered here. For the all-β proteins (class II, Fig. 2), only one out of the 50 hydrogen-bonding associations in the β sheets is between a pair of chains that run in the same direction (parallel) rather than in opposite directions (antiparallel); it is in prealbumin. For the α+β proteins (class III, Fig. 2) most of the β strands also form antiparallel rather than parallel associations (23 and five occurrences, respectively). On the other hand, the β sheets of the α/β proteins (class IV, Fig. 4) have many more parallel associations than antiparallel ones (64 and 21 occurrences, respectively). When the β strands are grouped together along the sequence and not separated by α helices (as in the all-β and α+β proteins), (ββ) and (βββ) folding units occur very often leading to antiparallel β sheets. When the β strands are separated by α helices along the sequence (as in the α/β proteins), (βαβ) and (βαβ)′ folding units occur most often leading to parallel β sheets.

Conclusions and implications

In the past the word domain has been used to describe substructures in protein molecules. This use derives from the observation of Phillips[58] who described the structure of lysozyme in terms of a series of "compact globular units". The word domain has also been used to describe the independent globular regions that can occur when a single polypeptide chain is formed by gene fusion or gene duplication (for example, the four domains of the immunoglobulin heavy chains). The use of the same word "domain" to define both types of protein substructure leads to confusion, and we suggest that the phrase 'folding unit' be used to define small assemblies of secondary structure segments that are adjacent in sequence and in van der Waals' or hydrogen-bonding contact with one another. The word domain is then reserved for large subassemblies that would be stable if the polypeptide chain connecting them to the rest of the protein molecule were to be cleaved[59]. Each domain has all the characteristics of a complete globular protein; often the different domains of a multidomain protein have different functions[60].

Our analysis of known protein structures using simplified topology/packing diagrams has shown that the observed arrangements of α helices and β strands are statistically very significant. The classification of 31 protein structures into four clearly separated classes has made it possible to identify common structural patterns, and has shown how the arrangement of segments of secondary structure along the sequence relates to three-dimensional properties such as parallel and antiparallel β sheets. Very often segments of secondary structure adjacent in sequence form a few well defined types of folding units. The high frequency of folding units in globular proteins is probably a result of the kinetic pathway of protein assembly rather than the stability of the final folded form. If certain segments of secondary structure existed with even marginal stability when the native conformation was unfolded, those segments that were close along the polypeptide chain would have a higher probability of diffusing together to form folding units[61]. If these folding units were then to associate rapidly to form the native conformation, which might be in a kinetically determined minimum of the free energy, the high frequency of such units in native proteins would be expected. The idea that segments of secondary structure close in sequence interact to form subassemblies which then associate to form the native structure has been used by Ptitsyn and Rashin to study the folding pathway of myoglobin[62]. Wetlaufer[63] used a similar kinetic argument to explain the domain structure of proteins. Other explanations of the high frequency of folding units in globular proteins do not depend on kinetic arguments: for example, the native conformation could be stabilised if its segments of secondary structure were to be connected up by short pieces of chain that do not cross one another. The chance probability that two evolutionary unrelated proteins have similar arrangements of secondary structure will be less than thought previously[1], for we have shown that all protein conformations are built up from the same three basic folding units and that the conformations fall into four well defined classes

Thus the picture of protein folding suggested by the final protein structure is as follows: pieces of secondary structure first diffuse together to form folding units that then associate to form the native structure, or in the case of the larger proteins, the domains which subsequently interact weakly to form the native structure.

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Identification of an enzyme in platelet microsomes which generates thromboxane A_2 from prostaglandin endoperoxides

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The microsomal fraction of horse and human platelets contains an enzyme which converts prostaglandin cyclic endoperoxides (PGG_2 or PGH_2) to a substance which is much more potent in contracting strips of rabbit aorta. This substance has the same characteristics as thromboxane A_2, and can be distinguished from other products of arachidonic acid metabolism by differential bioassay.

PIPER and Vane[1] have detected the release of a substance during anaphylaxis in isolated lungs from sensitised guinea pigs which they called "rabbit aorta-contracting substance" or RCS. The half life of RCS was <2 min at room temperature[2] and its release was blocked by aspirin-like drugs. RCS contracts all isolated vascular tissues tested from several species[2] and is released from lung tissue also by bradykinin, RCS–RF[2] SRS-C[3], arachidonic acid[3,4] and mechanical agitation or stimulation[2,4]. RCS is also generated by mechanical stimulation of rabbit spleen slices[5] and by incubation of arachidonic acid with rabbit spleen microsomes[5]. In addition, platelet aggregation is accompanied by the generation of an RCS[6–10]. RCS release has also been demonstrated *in vivo*, either by challenging sensitised guinea pigs with antigen or by infusion of bradykinin[4].

The release of RCS by the prostaglandin precursor, arachidonic acid, and the prevention of release by inhibition of prostaglandin biosynthesis led to the suggestion that RCS is an intermediate in prostaglandin biosynthesis[3,5]. A cyclic endoperoxide of arachidonic acid was postulated as the unstable intermediate in the biosynthesis of prostaglandins[11,12]. Endoperoxides have been generated and isolated after incubation of arachidonic acid with sheep vesicular glands[13,14]. The prostaglandin endoperoxides (now most frequently designated PGG_2 and PGH_2; Fig. 1) contract rabbit aorta, gastrointestinal and tracheal smooth muscle[15] and at low concentrations induce aggregation of platelets[10,16]. Aggregation induced by collagen, thrombin, adrenaline and arachidonic acid is also accompanied by release of endoperoxides[10,16,17]. The inhibitory effect of aspirin on the second phase of platelet aggregation[18] could therefore be due to inhibition of biosynthesis of platelet endoperoxides.

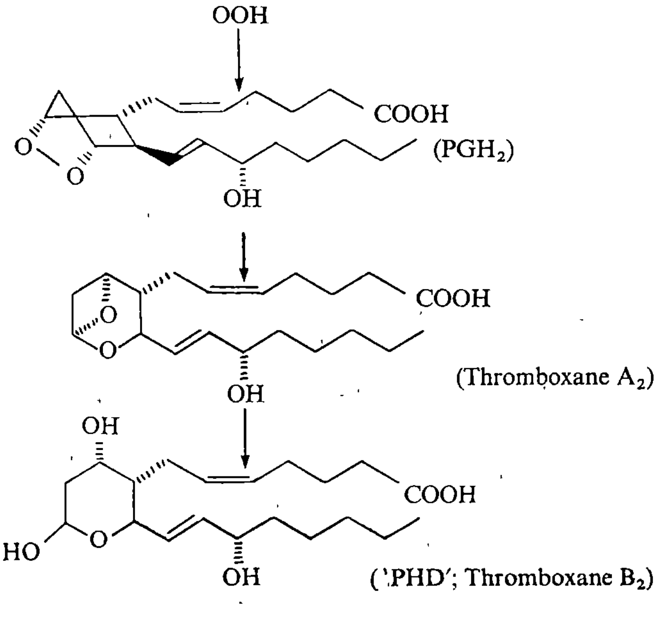
PGG_2 and PGH_2 are unstable, but their half lives are longer ($t_{\frac{1}{2}} \simeq 5$ min) than that of RCS[10,16]. Furthermore, quantitative analysis of the endoperoxide released from either guinea pig lung effluent or human platelets showed that only a minor part of the rabbit aorta-contracting activity was due to prostaglandin endoperoxides[10].

Samuelsson's group[19] has discovered a labile intermediate in the conversion of the endoperoxide PGG_2 into the hemiacetal derivative PHD (Fig. 1). They named the intermediate thromboxane A_2 (TXA_2) because of its structure and potent aggregating activity on platelets. This substance also strongly contracts rabbit aorta strips and its half life in aqueous solution is approximately 30 s. These properties of TXA_2 resemble very closely the properties of RCS, and it has been concluded that the activity of RCS is mainly due to TXA_2 (ref. 19).

Washed human platelets convert PGG_2 to TXA_2 (ref. 19). In the work reported here, we isolated from the same source and also from horse platelets an enzyme system which converts endoperoxides to a substance with the characteristics of TXA_2 and compared the relative contractile effects of endoperoxides and this substance on rabbit aorta.

Two litres of fresh citrated (3.8% w/v) blood from a horse were spun at 200*g* for 10 min to remove red blood cells. The

Fig. 1 Structures of some metabolites of arachidonic acid.



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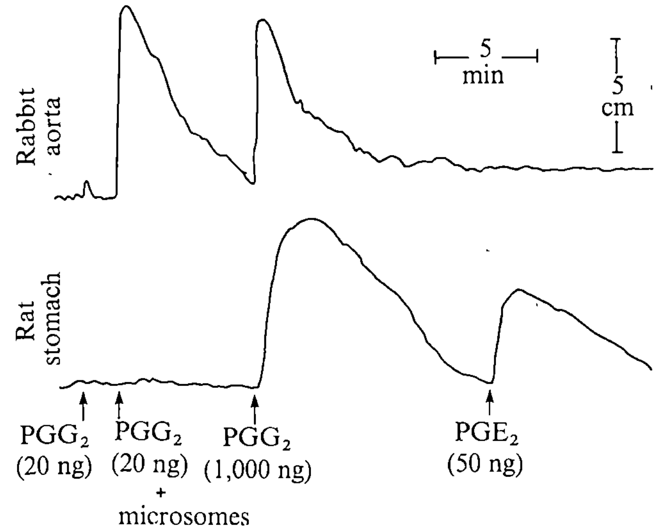


Fig. 2 Differential bioassay of rabbit aorta-contracting substances The treatment conditions are indicated at the bottom of the figure PGG$_2$ (200 ng) was added to 500 µl of Tris buffer (50 mM, *p*H 7 8) at 0 °C and a 50-µl sample (equivalent to 20 ng) was tested. Immediately after testing, horse platelet microsomes were added to the PGG$_2$ solution and 50 µl was tested 2 min later

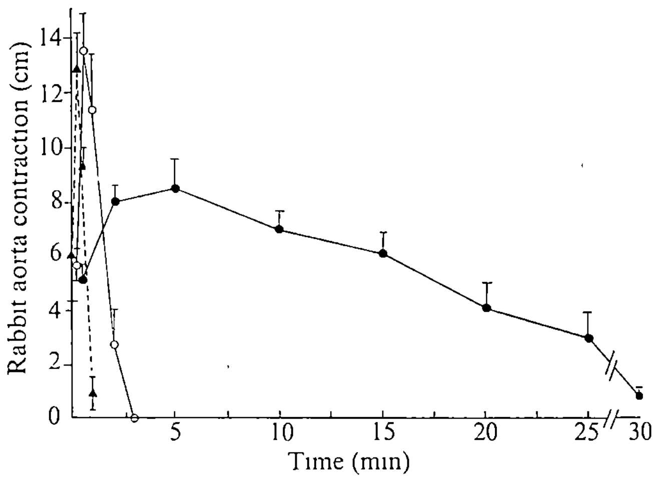


Fig. 3 Time course and temperature dependence of TXA$_2$ synthetase PGG$_2$ (500 ng) was added to 1 ml of Tris buffer (50 mM, *p*H 7 8) and incubated at 0 °C (●), 22 °C (○) or 37 °C (▲) before bioassay (50 µl) The values are the means (± s.e.) of four experiments at each temperature.

platelet-rich plasma was centrifuged (2,000*g*) for 15 min and the pellet of platelets was resuspended in 20 ml of 100 mM Tris (*p*H 7.5) buffer. The platelet suspension was subjected to freezing and thawing three times. Disrupted platelets were centrifuged at 5,000*g* for 15 min to remove cell debris. The supernatant was spun at 100,000*g* for 60 min The resultant pellet was washed and resuspended in 2 ml of 100 mM Tris (*p*H 7.5) buffer and homogenised.

Electron microscopy of the particulate fraction showed it to be composed of vesicles bound by single membranes 50–100 nm in diameter. Because of these characteristics, we refer to this fraction as microsomes. The protein content of the suspension used was 14.2 mg ml^{-1}, as determined by the method of Gornall *et al.*[20]. A similar procedure was adopted in one experiment in which a particulate fraction from 1 litre of fresh human blood was prepared.

Biological activity was assayed on a rat stomach strip[21] and a rabbit aorta[1] superfused at a rate of 10 ml min^{-1} with Krebs solution at 37 °C, containing a mixture of antagonists[22], to inactivate acetylcholine, 5-hydroxytryptamine, catecholamines and histamine, and also indomethacin (1 µg ml^{-1}) to inhibit the synthesis of prostaglandins by the assay tissues[23].

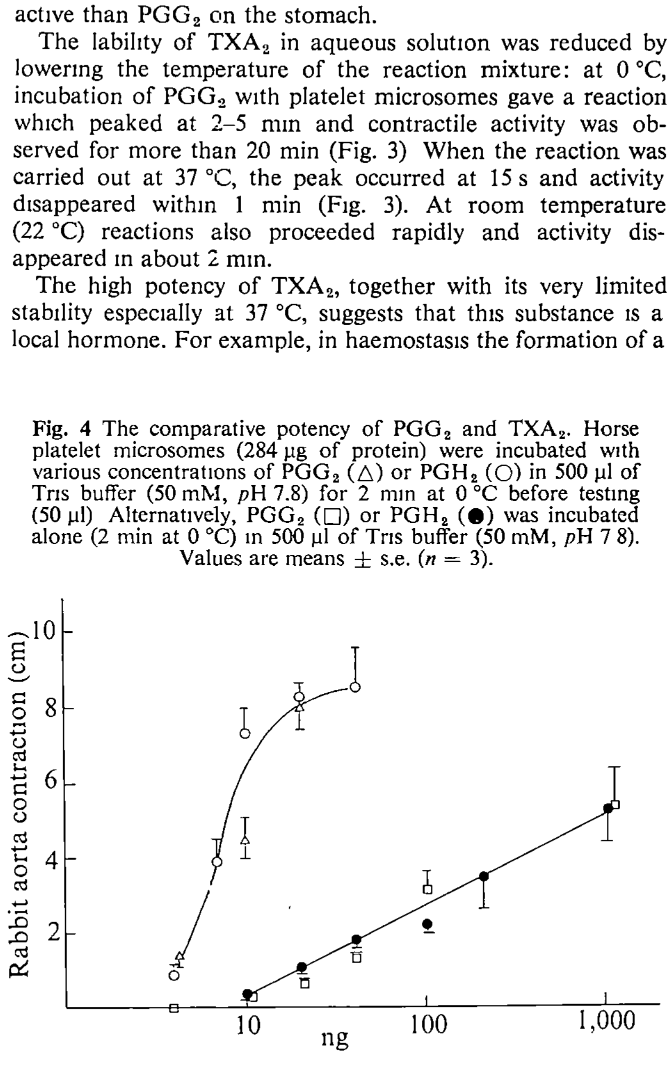
Application of PGG$_2$ or PGH$_2$ caused the assay tissues to contract in a dose-dependent manner. When PGG$_2$ was incubated with horse or human platelet microsomes and tested, there was a much greater contraction of rabbit aorta than was caused by PGG$_2$ alone (Fig. 2). Indomethacin (up to 25 µg ml^{-1}) did not affect the generation of the more potent compound. Similarly when PGG$_2$ was incubated with intact washed platelets, a powerful rabbit aorta-contracting substance (presumably TXA$_2$) was produced. But neither boiled platelet microsomes nor the 100,000*g* supernatant fraction of lysed platelets converted PGG$_2$ to the more potent compound. Furthermore, when PGG$_2$ had stood in aqueous solution at room temperature for 25 min, it no longer caused contraction of the aorta, nor did it yield an aorta-contracting substance when subsequently incubated with platelet microsomes. The substance generated from PGG$_2$ by platelet microsomes was highly unstable, with a half life at 37 °C of ~ 30 s. From these characteristics we assumed that the compound generated was TXA$_2$.

Figure 2 illustrates how the rabbit aorta and the rat stomach in a superfusion cascade can be used to distinguish between PGG$_2$, TXA$_2$ and PGE$_2$. PGG$_2$ (1,000 ng) caused both rabbit aorta and rat stomach to contract. A lower dose (20 ng) of PGG$_2$, that had little or no effect on these tissues before incubation with platelet microsomes, yielded sufficient TXA$_2$ to cause a larger contraction of the rabbit aorta than 1,000 ng of PGG$_2$—more than a 50-fold increase in potency. TXA$_2$, (unlike PGG$_2$) had no effect on rat stomach, however. On the other hand, PGE$_2$ (50 ng) produced a large contraction of rat stomach without affecting rabbit aorta. It was 3 to six times more active than PGG$_2$ on the stomach.

The lability of TXA$_2$ in aqueous solution was reduced by lowering the temperature of the reaction mixture: at 0 °C, incubation of PGG$_2$ with platelet microsomes gave a reaction which peaked at 2–5 min and contractile activity was observed for more than 20 min (Fig. 3) When the reaction was carried out at 37 °C, the peak occurred at 15 s and activity disappeared within 1 min (Fig. 3). At room temperature (22 °C) reactions also proceeded rapidly and activity disappeared in about 2 min.

The high potency of TXA$_2$, together with its very limited stability especially at 37 °C, suggests that this substance is a local hormone. For example, in haemostasis the formation of a

Fig. 4 The comparative potency of PGG$_2$ and TXA$_2$. Horse platelet microsomes (284 µg of protein) were incubated with various concentrations of PGG$_2$ (△) or PGH$_2$ (○) in 500 µl of Tris buffer (50 mM, *p*H 7.8) for 2 min at 0 °C before testing (50 µl) Alternatively, PGG$_2$ (□) or PGH$_2$ (●) was incubated alone (2 min at 0 °C) in 500 µl of Tris buffer (50 mM, *p*H 7 8). Values are means ± s.e. (*n* = 3).



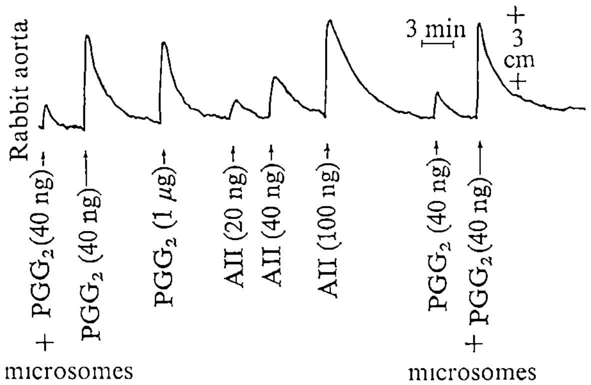


Fig. 5 Comparative potency of TXA_2 and angiotensin II (AII). Horse platelet microsomes (154 µg protein) were incubated with PGG_2 (200 ng) in 500 µl of Tris buffer (50 mM, *p*H 7 8) for 2 min at 0 °C before testing (100 µl equivalent to 40 ng PGG_2). Contractions to angiotensin II and PGG_2 alone are shown.

platelet plug should be facilitated when the lumen of the severed blood vessel is constricted The endoperoxide and TXA_2 generated by aggregating platelets could produce a profound vasoconstriction largely restricted to the area of the vessel immediately adjacent to the platelet plug. Vasospasm associated with coronary or cerebral infarction could be produced by potent vasoconstrictors generated during platelet clumping.

Drugs that inhibit endoperoxide generation (for example, indomethacin) or inhibit TXA_2 biosynthesis could reduce vasoconstriction as well as platelet aggregation and thereby prolong bleeding time.

PGH_2 was also converted to a more potent aorta-contracting substance by platelet microsomes. A quantitative comparison of PGG_2 and PGH_2 and their activation by microsomes is presented in Fig. 4. The dose–response curves, however, are not parallel and it is difficult to assign a relative potency. As Figs 2 and 4 show, 1,000 ng of PGG_2 or PGH_2 applied directly to the aorta matched the contraction produced by 20 ng of PGG_2 (or PGH_2) when incubated for 2 min at 0 °C with platelet microsomes. This observation underestimates the potency of TXA_2 because (1) at 2 min the contractile activity represented the resultant of synthesis and destruction of TXA_2, and no correction for destruction was applied and (2) the comparisons were made on the basis of the degree of the contraction of the aorta but the response to TXA_2 usually lasted longer than that to endoperoxide.

Detection of low concentrations of endoperoxides could be amplified substantially by adding platelet microsomes to convert PGG_2 or PGH_2 to the more potent TXA_2. Similarly, addition of platelet microsomes made it possible to discriminate between endoperoxides and TXA_2. For example, Willis[24] has described a "labile aggregation stimulating substance" (LASS) which aggregates platelets and is a potent aorta constrictor. If extracted LASS is an endoperoxide, platelet microsomes would be expected to enhance its contractile potency whereas the platelet enzyme would exert no effect on already formed TXA_2.

In a further experiment the activity of PGG_2 and TXA_2 in causing rabbit aorta to contract was compared with that of angiotensin II (Fig. 5). PGG_2 was less active (1/4–1/5th molar basis) than angiotensin II, whereas TXA_2 was at least as potent as angiotensin II. It is therefore conceivable that TXA_2, like angiotensin, will turn out to be a powerful vasoconstrictor in the body.

From these results we conclude that we have identified an enzyme in platelet microsomes which converts prostaglandin endoperoxides into the more potent and more unstable aorta constrictor, TXA_2. This has also been demonstrated directly by isolation of labelled thromboxane B_2 (Fig. 1) after incubation of ^{14}C-arachidonate with a particulate fraction from human platelets (unpublished results of P. Falardeau and B.S.).

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letters to nature

Pulsar spin down and cosmologies with varying gravity

IN this paper I show that some cosmologies with weakening gravity are in conflict with the measured spin down of the pulsar JP1953. If gravity is weakening, it must do so even more slowly than the limits set by radar ranging For group theory cosmologies with strengthening gravity, we also give a natural explanation for the lack of long period pulsars.

Certain very large dimensionless ratios of the fundamental constants of physics and astronomy exist. Dirac[1,2], in his large number hypothesis (LNH), has suggested that these ratios are interrelated and simple functions of cosmic time. Thus the gravitational constant G as measured by atomic standards varies with cosmic time as t^{-1} and the number of nucleons in the Universe varies as t^2. Two possibilities for the implied continuous creation of matter were recently considered by Dirac[3,4]: first, nucleons are created uniformly throughout space (additive creation) and second, that matter is created where it already exists in proportion to the amount existing (multiplicative creation). The LNH can be used to generate four distinct cosmologies: the early Dirac cosmology[1,2], and the additive and multiplicative creation of matter cosmologies[3,4], and the cosmology of Hoyle and Narlikar[5-7].

Recently a group theoretical analysis of isotropic and spatially homogeneous cosmological models has been presented by Malin[8]. This analysis strongly suggests that the mass of all particles varies inversely as the four-dimensional radius of curvature of the Universe. Some of the cosmological and astrophysical implications of this mass variation have been explored in papers by Malin[8-10] and Mansfield and Malin[11]. Although the genesis and implications of the G-changing and mass-changing cosmologies are quite different[11], here both types of theories are considered as just prescribing a time variation of the dimensionless gravitational coupling parameter $\gamma = Gm_p{}^2/\hbar c$. All other constants and coupling parameters remain constant, except in the case of additive and multiplicative creation, where the number of nucleons in the Universe, $N, \propto t^2$. In the G-changing cosmologies $\gamma \propto t^{-1}$ while a family of cosmological models based on the group analysis gives $\gamma(t_0)/\gamma(t_0) = 3H_0$. (I take H_0, Hubble's constant[12] $= 55$ km s^{-1} Mpc^{-1}).

For an arbitrarily relativistic, degenerate, zero temperature star, exact polytrope models can be constructed where the pressure and density are related by $p = K\rho^{(n+1)/n}$ where K and n are constant. For these polytropes[13]

$$\gamma N^{(n-1)/n} R^{(3-n)/n} = f_n \qquad (1)$$

where N is the number of nucleons in the star, R its radius, and f_n is a function of n only which varies by 14% over the full range of stable polytropes. ($n = 1.5$ for non-relativistic stars and $n = 3$ for extreme relativistic stars.) All contributions from rotation, magnetic fields, nuclear forces, and relativistic gravity are neglected—a good approximation for my purposes.

The moment of inertia of a spherical star can be written $I = km_pNR^2$ where for stable polytropic stars[14] $0.04 \leq k \leq 0.132$. This definition and equation (1) can be combined to yield

$$\frac{\dot I}{I} = \frac{2n\dot\gamma}{(n-3)\gamma} + \frac{3n-5}{n-3}\frac{\dot N}{N} \qquad (2)$$

where I neglect the very small variation of n and k with time.

For decreasing γ, I assume that the pulsar rotates as a solid body and that the main pulse period is the period of rotation. Then a firm lower limit to the spin down rate $\dot P/P$ can be found by setting all electromagnetic losses and thus external torques equal to zero. In the early cosmologies of Dirac[1,2] and Hoyle and Narlikar[5,7] this means that angular momentum is conserved and hence I/P is constant, whereas in the recent cosmologies of Dirac[3,4] for an isolated star $I/P \propto MVR$ where the tangential rotation velocity, V, of the star is held constant rather than angular momentum. When angular momentum is conserved[1,2,5-7] a maximum $P/\dot P$ is found for $n = 1.5$ (non-relativistic)

$$\left[\frac{P}{\dot P}\right]_{\max} = \left[\frac{I}{\dot I}\right]_{\max} = -0.5\frac{\gamma}{\dot\gamma} \qquad (3)$$

where I use the fact that in these cosmologies $\dot N = 0$.

When the velocity is held constant[3,4] again the maximum is found in the non-relativistic extreme where

$$\left[\frac{P}{\dot P}\right]_{\max} = \left[\frac{R}{\dot R}\right]_{\max} = \begin{matrix}\text{none (multiplicative creation)}\\ -\gamma/\dot\gamma \quad \text{(additive creation)}\end{matrix} \qquad (4)$$

This analysis does not provide any useful limit for $(P/\dot P)_{\max}$ in the case of multiplicative creation since then $\dot P/P$ can be positive, negative or zero (when $n = 2$ 0) depending on the mass, and thus n, of the star. Equations (3) and (4) give instantaneous maxima—between 'glitches' for example.

The results of equations (3) and (4) are summarised in Table 1. From this it can be seen that the cosmologies of Dirac[1,2] and Hoyle and Narlikar[5-7] give, in the limit of zero electromagnetic losses, a $(P/\dot P)_{\max} < [8.6 \pm 1.3(2\sigma)] \times 10^9$ yr, the measured value for JP1953 (ref. 15). In fact, for any cosmology that conserves angular momentum, equation (3) with JP1953 implies that when $\dot\gamma < 0$ then $-\dot\gamma/\gamma \leq [5.8 \pm 1(2\sigma)] \times 10^{-11}$ yr^{-1} independent of H_0. This restriction on decreasing γ is slightly better than the restrictions provided by radar ranging (I. I. Shapiro, personal communication).

The values in Table 1 are derived neglecting relativistic gravity. This can easily be justified by noting that the maximum mass that can be supported against gravity by a degenerate gas scales[13] as $M_{\max} \propto \gamma^{-3/2} \propto t^{3/2}$. Thus old pulsars must be of very low mass and therefore relativistic gravity is unimportant. For a pulsar like JP1953, if $\dot\gamma/\gamma < 0$ its true age is actually greater than 0 $5P/\dot P = 4.3 \times 10^9$ yr as predicted by the standard ($\gamma =$ const.) spin down theory. Conservatively estimating its age at 4.3×10^9 yr, the maximum pulsar mass at its birth normalised by $M_{\max}(t_0)$ is seen to span the range

$$0.09 \leq M_{\max}(t_0 - 4.3 \times 10^9 \text{ yr})/M_{\max}(t_0) \leq 0.76$$

depending on which cosmology is considered. Thus it is clear that relativistic gravity is unimportant for old pulsars. In addition, the small effects of relativistic gravity would be overwhelmed by any reasonable spin down because of electromagnetic losses.

For increasing γ according to the group analysis $\dot\gamma > 0$, then $\dot I < 0$. This makes the pulsar spin up—counteracting the spinning down from electromagnetic losses. For either a dipole magnetic field inclined at an angle to the spin axis or for a standard pulsar magnetosphere model the loss rate $\simeq -B^2R^6\omega^4/c^3$, where B is the surface component of the dipole field at the polar cap[16]. Then the proper equation for the case when $I(t)$ varies according to equation (2) with $\dot N = 0$ is

$$\frac{\mathrm{d}P^2}{\mathrm{d}t} = \frac{8\pi^2B^2R^6}{c^3I} - \frac{4P^2n\dot\gamma}{(3-n)\gamma} \qquad (5)$$

Notice that $\dot P \rightarrow 0$ for a critical period given by

$$P_c = \left[\frac{2\pi^2B^2R^6(3-n)}{3c^3InH(t)}\right]^{1/2} \qquad (6)$$

where $\dot\gamma/\gamma = 3H(t)$ as prescribed by the group theory analysis. P_c is the period at which the spin down from electromagnetic losses is just compensated for by the spin up because $\dot I < 0$. Of course, since $\dot P \rightarrow 0$ there is no limit for $(P/\dot P)_{\max}$ in this cosmology.

$R^3/I^{1/2}$ can vary by almost an order of magnitude for the full range of stable neutron stars[16]. Models[17] for the genesis of neutron stars suggest, however, that they should be formed with a mass near the Chandrasekhar limit $\sim 1.3M_\odot$. On the basis of these arguments Ruderman and Sutherland[16] adopt a model in which $M = 1.25M_\odot$, $R = 8.1$ km, and $I = 6.24 \times 10^{44}$ gm cm^2. In these conditions $n \simeq 2$. Then equation (6) yields $P_c \simeq 5(B/10^{12}$ gauss). Thus for very reasonable pulsar parameters $P_c \simeq 5$ s with a factor of ~ 5 uncertainty because of the permissible range in pulsar parameters. Therefore when $\dot\gamma/\gamma = 3H_0$ as predicted by the group analysis, the pulsars should tend to a limiting period of $P_c \simeq 5$ s which is consistent with $P \leq 3.74$ s for the 147 known pulsars (Y. Terzian, personal communication). Thus, the increasing γ cosmology is consistent with the pulsar data and offers a natural explanation for the

Table 1 Maximum values of $P/\dot{P}$ for the decreasing γ cosmologies in the limit of zero electromagnetic losses. The measured $P/\dot{P} = [8.6 \pm 0.8(1\sigma)] \times 10^9$ yr for JP1953

Cosmology	t_0	$(P/\dot{P})_{\max}$	$(P/\dot{P})_{\max}$ (yr)
Early Dirac	$\frac{1}{3}H_0^{-1}$	$t_0/2$	3×10^9
Hoyle–Narlikar	$\frac{1}{2}H_0^{-1}$	$t_0/2$	4.4×10^9
Dirac additive creation	H_0^{-1}	t_0	1.8×10^{10}
Dirac multiplicative creation	H_0^{-1}	----	----

lack of long period pulsars independently of any details of pulsar magnetospheres.

An exact solution of equation (5) requires a numerical integration and is dependent on the details of the cosmological model through $H(t)$ which increases slowly with time[8]. Considerable insight can, however, be obtained by letting $4\pi\dot{\gamma}/\gamma^{-1}(3-n)^{-1} \simeq 15H_0$ in the last term in equation (5). This overestimates the term at early times when it is unimportant and underestimates it slightly for $t > t_0$. When the magnetic flux through the poles is conserved the first term in equation (5) is constant. Then

$$(P/P_c)^2 = 1 - \exp(-15H_0\tau) \qquad (7)$$

where τ is the time since the pulsar was born. For $15H_0\tau \ll 1$, equation (7) reduces to the standard expression for $P(\tau)$. The effect of an increasing γ is only important for $\tau \gtrsim 5\times10^8$ yr. Thus, only a small percentage of the oldest pulsars in the Galaxy would approach the limiting condition $\dot{P} = 0$ at $P = P_c$. For JP1953 the age implied by equation (7) would be $\tau = 1.8\times10^9$ yr rather than $\tau = 4.3\times10^9$ yr on the standard theory.

The above criticism of the G-changing cosmologies, derived on the basis of zero electromagnetic losses, is independent of the vagaries of magnetospheric models and loss rates. The model used to find $(P/\dot{P})_{\max}$ when $\gamma \propto t^{-1}$ is quite adequate for the old low mass pulsars considered. Therefore the values in Table 1 should be a firm upper limit for $P/\dot{P}$ with the major uncertainty coming from the uncertainty in H_0. Of course, this analysis also applies to spinning white dwarfs.

JP1953 could be a much more exotic object than assumed or could participate in some very implausible orbital effects. These possibilities have a very small but finite probability and will be examined in detail in a forthcoming paper.

The critical period, $P_c \simeq 5$ s, derived from the group theory result with $\dot{\gamma}/\gamma = 3H_0$, is not strongly dependent on relativistic gravity either since $P_c \propto (I/\dot{I})^{1/2}$. The uncertainties here come from the fairly large range in permissible pulsar parameters and there seems to be no clear way to narrow the range.

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ANS observations on the X-ray burster MXB1730–335

A NEW type of time variability of cosmic X-ray sources ('bursters') was discovered from Astronomical Netherlands Satellite (ANS) observations on the source 3U1820–30, associated with the globular cluster NGC6624 (refs 1, 2). These X-ray bursts are characterised by a rapid rise in flux (~ 0.5 s) to a relative intensity comparable to the Crab Nebula, followed by an exponential decay at a time scale of ~ 10 s. Certain characteristic features of the bursts, in particular the shape and spectral hardening, are consistent with a model where the X-ray emitting region is situated in a cloud of hot gas near a massive black hole (~ 1,000$M_\odot$) (ref. 3). On March 14, 15 and 16, 1976 the ANS spacecraft was pointed in the direction of the remarkable new X-ray burster MXB1730–335 discovered by SAS-3 (ref. 4) in the constellation Scorpius at 5° from the galactic centre in the galactic plane. The source is characterised by rapidly repetitive X-ray bursts of varying intensity. The energy in a given burst is approximately linearly proportional to the time interval to the next burst[5]. We report here an improved position of this source and give the energy spectrum from data obtained in four observations for which quick-look data are available. An upper limit for the steady source level in between the bursts is obtained.

In Fig. 1 the counting rate histogram is shown for two observations, from the Utrecht experiment SXX, in the energy range 1–7 keV. Spectral information is available per second in the 1–7 keV range (SXX) and every 4 s in the 1.3–30-keV range for the Cambridge experiment HXX. The steady source intensity in these data is < 1 count s^{-1} or ~ 15 Uhuru counts s^{-1} ($\lesssim 3.10^{36}\times d^2_{10}$ erg s^{-1} in the range 1–7 keV, where d_{10} is the distance (to the source) in units of 10 kpc). We observed the source when it was emitting bursts at an almost constant peak intensity of 16 counts s^{-1} on March 14.639 (Fig. 1*b*) and 12 counts s^{-1} on March 14.173 (Fig 1*a*) or ~ 0.2 and 0.15 times

Fig. 1 Two examples of counting rate histogram (Utrecht SXX experiment) of the burster source MXB1730–335 in the energy range 1–7 keV *a*, March 14.173; *b*, March 14.639. Integration time (1 bin) is 1 s.

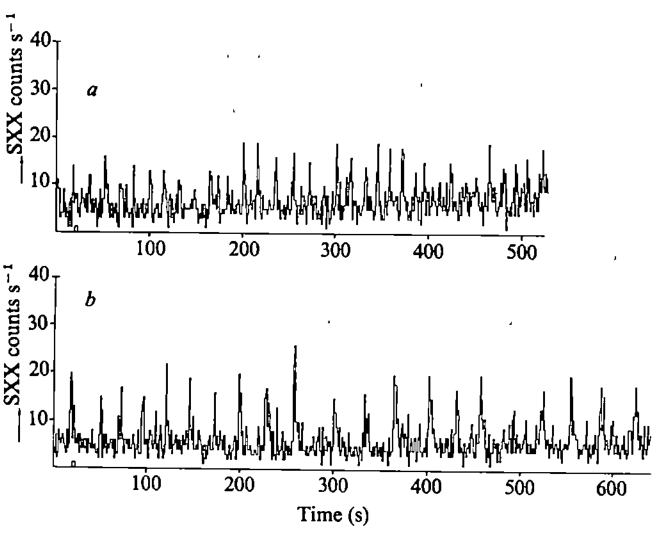

Table 1 Data on X-ray bursters

Source	Discovered by	Position (1950.0)	Area of error box (degree)	Remarks	Reference
NGC6624	ANS	18 h 20.5 min −30°23′	0.033	Bursts at intervals of ~ 0.18 d	1, 2, 5, 14
In Norma	Vela-5	16 h 07 min −53°	~ 10		12
9 events A through I	Vela-5		~ 100		12
MXB1743−293	SAS-3	17 h 42.6 min −29°16′	0.01	Bursts at intervals ~ 0.55 d	16–18
MXB1742−297	SAS-3	17 h 41.7 min −29°40′	0.01	Bursts at intervals ~ 1.46 d	16–18
NGC1851	Uhuru	05 h 12.4 min −40°05′	5		15
In Aquila	SAS-3	19 h 29 min +8°	~ 150		23
MXB1730-335	SAS-3	17 h 30 min 08 s −33°21.5′	0.0022	Frequent bursting, associated with globular cluster	4–9 this paper
MXB1728-34	SAS-3	17 h 28 min 29 s −33°48′	0.0035	Bursts at intervals ~ 0.20 d	4, 5
In Puppis	SAS-3	07.6 h −50°	~ 170		24

the intensity of the Crab Nebula. The rise time of the bursts is $\lesssim$ 1 s, a typical characteristic of the bursters. The duration of the bursts varies and is typically a few seconds. The average time between the bursts is 26 s and 16 s, respectively, and the total energy per burst (1–7 keV) is ~ $10^{38}d^2{}_{10}$ erg and ~ $8\times10^{37}d^2{}_{10}$, respectively. This is consistent with the relationship found by Lewin *et al.*[5] for the energy per burst and the time separation between the bursts over a somewhat different energy range. We integrated 20 bursts to obtain a signal-to-noise ratio sufficient for a determination of the position and the spectral parameters.

The position of the source is obtained by comparing counting rates of the various X-ray instruments aboard ANS. The two 3° × 10′ FWHM field of view collimators of the Cambridge experiment HXX, which are mutually offset with 3.7′ have a count-rate ratio for 20 integrated bursts of 2.20 ± 0.16. This determines the declination of the source (90% confidence level) to be $2.5\pm1'$ north of the pointing direction used, which was the most probable SAS-3 position[6], supplied by W. Lewin before publication. Hence $\delta(1950.0) = -33°22.5'\pm1'$. The ratio of the counts seen in the HXX and SXX detectors confines the source to the central position of the field in right ascension ($\pm30'$). The position thus obtained[7] is plotted in Fig. 2, together with the SAS-3 position. The region of intersection ($2'\times3'$) of the possible positions for the source MXB1735−335 reveals a faint, highly reddened star cluster, probably globular, with coordinates α = 17 h 30 min 08 ± 01 s, $\delta(1950.0) = -33°21'25$ $\pm10''$ (ref. 8). Also the position found with the rotation modulation counters aboard Ariel V ·(ref. 9) is consistent with the cluster being the source MXB1730−335.

The integrated X-ray burst spectrum, obtained by adding 20 bursts after subtracting the background before and after each burst, is characterised for an exponential fit by kT of 10^{+3}_{-2} keV and a photoelectric absorption, from a hydrogen column density of 5^{+2}_{-1} 10^{22} atoms cm^{-2} (using Brown and Gould[10] abundances). (Quoted errors are 1 s.d.) This value for the hydrogen column density N_H taken together with the estimated visual absorption[8] for the globular cluster A_V = 15 mag, matches quite well with the gross average for the Galaxy for this ratio. $N_H/A_V = 2.2\times10^{21}$ cm^{-2} mag^{-1} as derived in ref. 11. This implies that the low energy absorption cutoff of the burster is consistent with the interstellar absorption found for the globular cluster. Separate spectra for the peak and the decay phase of the bursts show evidence for a spectral hardening during the burst. This feature will be studied further, when all (production) data for this source will be available.

We have therefore observed the same rapidly repetitive X-ray burster that MIT reports[5], based on positional agreement and general X-ray characteristics. The improved positional error box of this source ($2'\times3'$) contains a globular cluster and provides thus another example of an X-ray burster that may be related to globular clusters. The consistency between the interstellar absorption measured from the burster and the optical extinction measured from the cluster further provides evidence for the identification.

At about the same time of the ANS discovery of an X-ray burster in NGC6624, a report from 15 months of operation of the two Vela-5 satellites showed 20 X-ray bursts of duration > 2 s and < 128 s (ref. 12). Ten of these are consistent with one position in the constellation Norma and lie close to a transient X-ray source found by Uhuru (ref. 13). Subsequently many more bursts have been found in data from the SAS-3, Uhuru and Ariel V satellites. Clark[14] found 11 bursts in SAS-3 data from a region of the sky of 12° × 12° containing the globular cluster NGC6624, exhibiting the same characteristic time structure and spectral hardening as the ANS bursts and thus most probably originating from the same globular cluster. The bursts show a mean time separation of 4.3 h with a 4% r.m.s. deviation. It is probable that during SAS-3 observations in January 1976 on 3U1820−30, when the source was twice the maximum Uhuru intensity no X-ray bursts were observed[5], suggesting indeed that the feature of X-ray bursts in NGC6624 is related to the intensity level of 3U1820−30.

In the old Uhuru data an X-ray flare of duration > 15 s and < 6 min was found[15] in a region of 0.5° × 10° containing the globular cluster NGC1851. Although the complete time structure, in particular the rapid rise time, could not be detected from a spinning satellite, the observed spectral hardening during the event indicates that one is probably dealing indeed with the same type of X-ray bursts.

Two sources of X-ray bursts, designated MXB1743−293 and MXB1742−297, were found by SAS-3 within a degree of the galactic centre [16-18]. The bursts show a mean time separation of 1.46 and 0.55 d, respectively. The bursts from MXB1742−297 seem to show in most cases a double pulse structure at the higher energies (8–18 keV). A summary of the newly discovered bursting X-ray sources is given in Table 1

The nature of the bursting X-ray sources thus seems to be quite different from sources identified so far as binary X-ray stars The importance of this was already stressed by Grindlay *et al.*[2]. At least three of the dozen now known bursters are identified with globular clusters (NGC6624, NGC1851 and Liller's globular cluster). Three others (MXB1743−293, MXB1747−297 and MXB1728−34) originate in the central region of the Galaxy and could very well be related to completely obscured globular clusters. The remaining bursters have

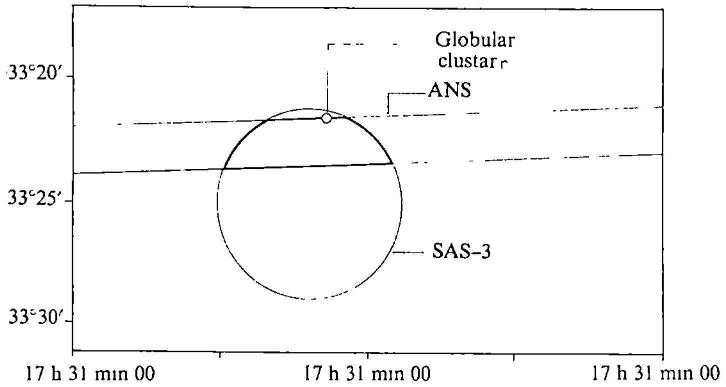

Fig. 2 Positions obtained by various satellites of the X-ray burster MXB1730−335. In the intersection of the error boxes a newly discovered globular cluster is present.

positions that are too poorly defined to make any definite conclusion regarding identification. Two bursters (NGC6624, NGC1851) are also known X-ray sources (3U1820−30 and MX0513−40) that belong to a group of seven sources associated with globular clusters.

The correlation between the intensity level of 3U1820−30 and the existence of X-ray bursts in that source, indeed suggest a physical relationship between the two. Several authors have pointed to the intrinsically different nature of globular X-ray sources. This follows, for example, from the ratio of the X-ray luminosity, which is one of two orders of magnitude larger than the corresponding ratio for the entire Galaxy or the galactic bulge[20] The majority of these seven X-ray sources are associated with globular clusters that have high central escape velocity and are centrally condensed. This has led various authors[21,22] to suggest that the X-ray producing mechanisms somehow are related to the existence of massive remnants of evolved stars and possibly with massive black holes of ~ 100–1,000 $M_\odot$. This is consistent with such a massive black hole if the decay phase of the burst is interpreted as Compton scattering from a hot cloud around the hole.

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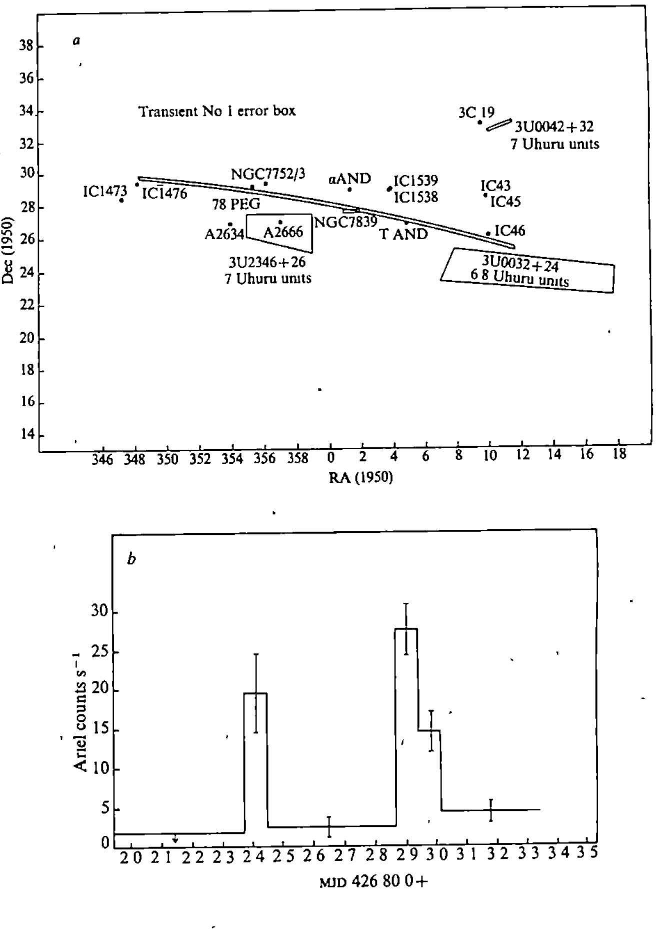
Two short lived X-ray transients at high galactic latitude

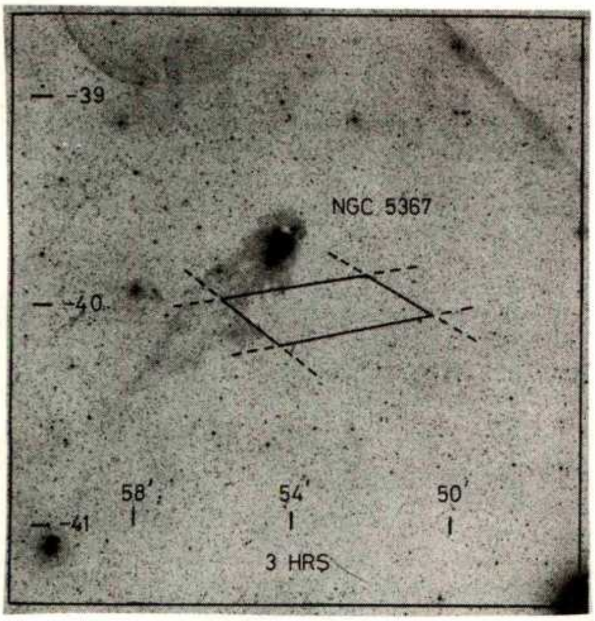
UNTIL recently, sources known to exhibit transient behaviour were restricted to a region close to the galactic plane. Now, extended observations at high galactic latitudes by the Ariel V, ANS and SAS-3 X-ray detectors have revealed transients with an impressive variety of temporal behaviour. Reporting the first such transient Ricketts *et al.*[1] described how A1103+38 ($b^{II} \simeq 65°$) increased in intensity by an order of magnitude over a period of between 10 and 28 d with an additional 'flare' which lasted ~ 1 d. In contrast Rappaport *et al.*[12] observed a spectrally hard transient MX2346−65 ($b^{II} \simeq -51°$) which lasted between 45 s and 2,200 s (ref. 2) and was about as intense as the Crab Nebula. In the error box of A1103−38 the radio source B2 1101+38, whose optical counterpart MK421 may be a BL Lacertae object, could be responsible for the X-ray transient. As there are no obvious optical candidates with which to associate MX2346-65, Rappaport *et al.* suggest sporadic mass transfer on to a white dwarf from a faint, variable dM class star. We here report two transients at high galactic latitudes observed by the Leicester sky survey experiment on Ariel V whose time scales, of the order of hundreds of minutes, are intermediate between those discussed above.

Besides their intrinsic interest, it seems possible that these various high latitude transients have some bearing on the less well determined, mainly unidentified high latitude X-ray sources in the third Uhuru catalogue, some of which may also be associated with relatively nearby galactic objects.

The first new Ariel V transient source, observed on September 27, 1975, was in a region of sky close to Uhuru

Fig. 1 *a*, Error box (90% confidence in width, full collimator dimension in length) for the transient A0000+28 of September 27, 1975 *b*, Light curve of transient A0000+28 for source at centre of error box. Error bars are ± 1σ from counting statistics.





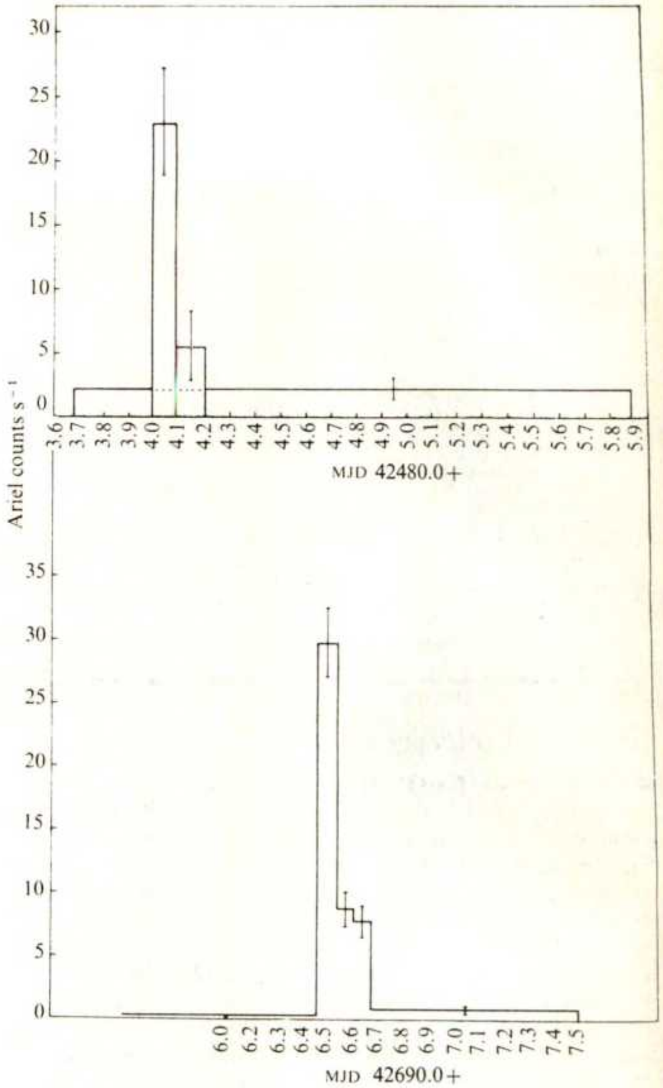


Fig. 2 *a*, Superposition of error boxes of A0353−40 transients of March 12 and October 1, 1975 on Sky Survey plate. Coordinates are 1950. Photograph reproduced by courtesy of SRC Schmidt Telescope Unit. *b*, Transient A0353−40 light curves assuming common origin of X-ray emission near NGC5367. Error bars are ± 1σ from counting statistics.

sources 3U2346+26, 3U0042+42 and 3U0032−24. The long thin error box (Fig. 1*a*) which results from a single observation of the transient, contains no obvious optical or radio candidate for association except for T And, an M3 type, Mira variable, whose visual magnitude varies between 7.7 and 14.3 with a period of 280.91 d (ref. 3). Fabian, Pringle and Webbink[4] discussed the possibility of transient X-ray emission from Mira variables in relation to the Cen Xmas source A1118−61. The light curve of the September 27 transient is shown in Fig. 1*b*, corrected for an assumed source position at the centre of the error box. The light curve shows an initial peak (related to precursor peak common to the galactic plane transients[5]?), followed by a main peak lasting about three satellite orbits (~ 5 h).

An unsuccessful search through Ariel V data for other appearances of the transient gave a good opportunity to investigate the nearby 3-U sources. If still at their catalogued strength (~ 6 Uhuru units in each case) each should have been observed with high significance (~ 5σ) at least twice in the Ariel V data. The non-observation of any of the sources places the strengths at no more than 2 Uhuru units and suggests a variability that makes unlikely the association of 3U2346+26 with the Abell clusters A2666 or A2634 (ref. 6).

The second new transient was noticed in Ariel V quick look data on October 11, 1975, and found to be in the region of 3U1439−39 and Cen-A. Examination of earlier sky survey data for this region revealed a transient ~ 200 d earlier. From the number of high latitude transients which have been observed by the Ariel V sky survey experiment (five since launch), the probability that what has been observed comes from 2 different transients is only 10^{-3}. The error boxes intersect (Fig. 2*a*) and both light curves in Fig. 2*b* have been calculated by normalising the counts as from an object at the centre of the combined error box. Their similarity with respect to amplitude and time scale further suggest that the same transient has been observed twice. Neither the error box, nor the field close by contain any reported variable stars[3] or unusual extragalactic objects; all the stars in the error box are fainter than $M_v = 9$. There is an $M_v = 6.3$ double star **18936** in Aitken's catalogue at 3 h 58 min, −39.9°.

Near the edge of the transient error box is the impressive, bright reflection nebulosity NGC5367. From a bright diffuse head containing embedded stars a diffuse tail extends nearly one degree towards the galactic plane. The cometary appearance of NGC5367 and similar objects in the region of the Gum, and possibly Orion, nebulae have earned them the description 'cometary globules'[7]. However it has been initiated in such isolated clouds, star formation clearly is taking place in NGC5367, the obvious embedded stars being of mid to late B type[8]. Such a structure should be expected to contain also possibly T Tauri stars[9] and analogously with the Orion and Taurus T associations also UV Ceti flare stars. Predictions of X-ray flare intensities from UV Ceti stars have already been made by scaling up from the stellar radio emission during a flare using the ratio between X-ray and radio fluxes observed in solar flares[10]. To date, no X-ray flares from UV Ceti stars have been detected[11] though long term correlated radio, optical and X-ray observations have been made (C. J. Crannell *et al.*, unpublished) and are being analysed. For an $M_v = 1$ flare on UV Ceti the most optimistic X-ray flux in the range of the

Ariel V sky survey experiment is ~ 10^{25} J (ref. 10) which is to be compared with a peak emission of ~ 10^{27} J from NGC5367 assuming its distance to be 300 pc (ref. 8). If NGC5367 is the origin of the transient X-ray emission it would repay study for significant variability in other parts of the spectrum.

I am grateful for discussions with Dr P. W. J. L. Brand concerning NGC5367 and for his provision of an overlay for Fig. 2*a*. The Ariel V project is supported by the SRC.

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Statistical interpretation of enthalpy–entropy compensation

Typically, enthalpy and entropy estimates from kinetic and equilibrium data are highly correlated, varying in a linear fashion with one another (enthalpy–entropy compensation effect). This effect can be readily explained in most cases simply as a statistical or data handling artefact. The statistical analysis presented here reveals three novel insights. First, the enthalpy and entropy parameter estimates are highly correlated, such that estimated correlation coefficients > 0.95, say, do not imply chemical causation. Second, enthalpy and entropy estimates are distributed by experimental and measurement errors in elliptical probability regions that are very elongated and appear as lines. The slope of such lines is the harmonic mean of the experimental temperatures. Third, estimates of enthalpy and free energy at the harmonic mean of the experimental temperatures are not statistically correlated, so any observed structured variation between these parameter estimates arises from the chemical effect alone. Note that, since the thermodynamic potentials are interrelated by the Maxwell relationships, a correlation between any two potentials can be transformed to give the corresponding correlation between any other two. We now discuss these results to resolve a number of issues concerning a much disputed data set.

Many linear enthalpy–entropy data sets have been catalogued for which the correlation coefficient > 0.95 (refs 1, 2). Implicit in such efforts is the hope that the high correlations imply the detection of a true chemical effect. For data on the oximation of alkyl thymyl ketones[3], for example, the correlation coefficient is 0.9724 and without the methylated compound it is 0.9988. It is not surprising, then, that these data have been rather widely discussed as perhaps displaying a linear compensation relationship[1,2,4–6]. Since the data were taken over a relatively narrow temperature range (30, 35 and 40 °C) the estimates are highly correlated. In fact the correlation between parameter estimates, even in the absence of any chemical compensation effect, is

$$\rho = \frac{\mathrm{Cov}(\Delta\hat{H}^{\neq}, \Delta\hat{S}^{\neq})}{[V(\Delta\hat{H}^{\neq})V(\Delta\hat{S}^{\neq})]^{\frac{1}{2}}} = \frac{\Sigma 1/T}{[n\Sigma(1/T)^2]^{\frac{1}{2}}} = 0.99991$$

Thus one cannot infer that the high observed correlation between enthalpy and entropy parameter estimates is not just a result of the deviations caused by errors. That the enthalpy and entropy estimates should be so highly correlated is displayed in Fig. 1. The measure of enthalpy is the slope of a regression line on an Arrhenius or van't Hoff plot. And the measure of entropy is the intercept at $1/T = 0$. Thus experimental or measurement errors will cause a slightly imprecise regression line with an error in the intercept measure 'compensating' the error in the slope measure. The intercept measure which is independent of slope deviations, however, is the one at the arithmetic mean of the independent variable, $1/T$, the inverse experimental temperature. Thus a useful reformulation of the Arrhenius equation is one that is centred about the average inverse experimental temperatures $\langle 1/T \rangle$

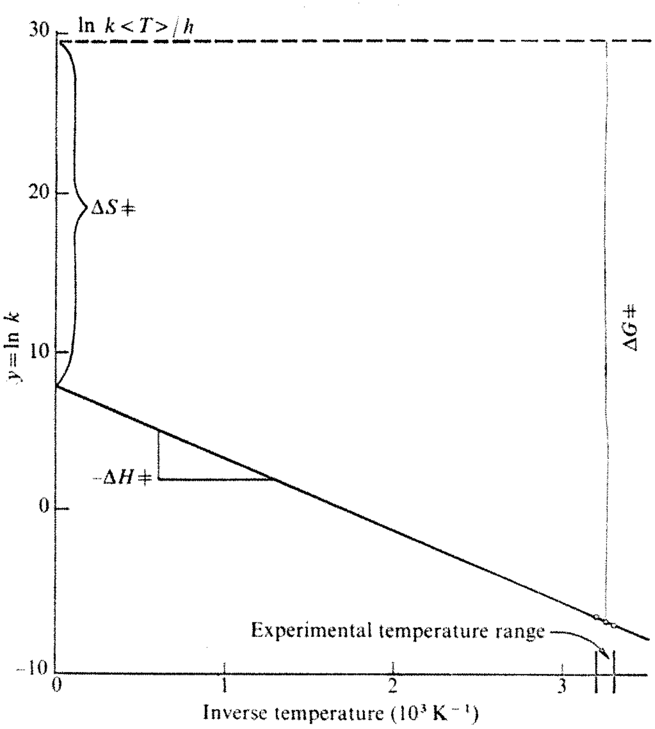

Fig. 1 Geometric interpretation of the parameter estimates. The indicated lengths are proportional to $\Delta G^{\neq}$ and $\Delta S^{\neq}$ and the indicated slope is proportional to $-\Delta H^{\neq}$. These data for the oximation of methyl thymyl ketone[6] indicate a strong dependence of the intercept estimate on the slope estimate because the data were taken over a very small temperature range far from the origin. The three data points are designed by dots.

$$\ln k = \ln A - E/RT = (\ln A - E/RT_{\mathrm{hm}}) - E/R(1/T - \langle 1/T \rangle)$$

Since this new intercept estimate is uncorrelated with the slope estimate, the correlation between enthalpy and free energy estimates at $T = T_{\mathrm{hm}}$ is zero

$$\rho_{\Delta\hat{H},\,\Delta\hat{G}_{T_{\mathrm{hm}}}} = 0$$

In particular the correlation between enthalpy and free energy estimates for the alkyl thymyl ketones is −0.2273 for the entire data set and 0.0770 for the data without the methylated compound. Since these correlations are not at all significant with just 7 and 6 data pairs, respectively, we conclude that no significant correlation between thermodynamic parameters is detected and the observed correlation in the enthalpy–entropy plane arises from the statistical propagation of errors as a data-handling artefact.

Much confusion has existed over how to estimate a line from data plotted in the enthalpy–entropy plane. To illustrate this, the thermodynamic parameter estimates for the oximation of alkyl thymyl ketones were plotted in Fig. 2 along with the corresponding 50% confidence regions. Since both the form of the Arrhenius equation and the transformed Arrhenius equation given above are linear, the confidence regions are elliptical. In particular, the ratio of major to minor axes in the enthalpy–entropy plane is 23,252:1. Thus the distribution of data is well characterised by merely the major axis of such a region, which is determined from a canonical analysis to be

$$\Delta H = T_{\mathrm{hm}}\Delta S + \Delta\hat{G}_{T_{\mathrm{hm}}}$$

and which differs from the postulated chemically caused line

$$\Delta H = \beta\Delta S + \Delta G_{\beta}$$

by the value of the slope parameter β, the compensation or isokinetic temperature[1,2,7,8]. The compensation temperature is commonly determined by least squares. The authenticity of a chemical compensation has been questioned when the value of the compensation temperature approaches that of the experimental temperatures[1,5,6,9–12]. We maintain that since the two relationships differ only in the slope parameter it is sufficient to test the value of that parameter against the harmonic mean of the experimental temperatures to determine if a chemical effect is detectable beyond the statistical compensation line. Furthermore, in the limit that the ratio of axes in $\Delta H - \Delta S$ coordinates is very large, lack of fit to the statistical compensation line is just the error in the estimate of $\Delta G_{T_{\mathrm{hm}}}$, which is distributed with zero mean and constant variance, justifying application of least squares to estimate a slope for comparison with T_{hm}. For the oximation of thymyl ketones, a 95% confidence interval for β is (226, 401) and without the methylated compound is (286, 328) where T_{hm} is 308 K. Thus no chemical effect was detected, as shown previously by the correlation analysis.

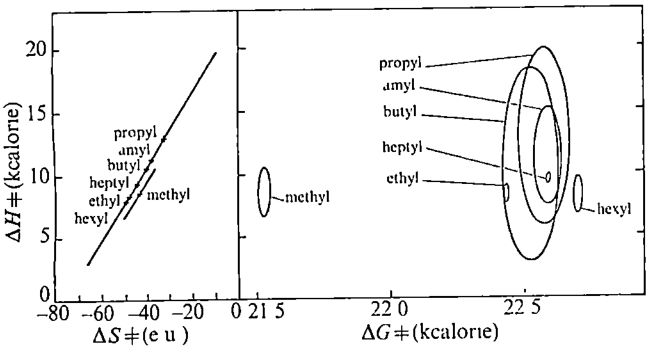

Fig. 2 The 50% joint confidence regions for the oximation of alkyl thymyl ketones[6]. The $\Delta H^{\ddagger}-\Delta S^{\ddagger}$ ellipses are so narrow that they appear as lines. Departure of the methylated compound from a common $\Delta G^{\ddagger}$ value causes it to fall off the statistical compensation 'line' between $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ estimates Notice that $\Delta G^{\ddagger}$ is estimated more precisely than $\Delta H^{\ddagger}$. All values were calculated for $T = T_{\mathrm{hm}} = 308.1$ K.

We have also tested 37 other data sets from several reviews[1,2,5,6,8,9,13] and for only three of these was the hypothesis rejected that the observed compensation pattern can be explained as an artefact. Only data sets that were thermally consistent, allowing computation of a unique T_{hm}, were tested.

From this analysis we conclude that high correlations of enthalpy–entropy estimates usually do not reflect any chemical causality but correlations between enthalpy-free energy estimates evaluated at T_{hm} do imply chemical causality. Least squares regression of enthalpy estimates on entropy estimates is only useful to determine if a chemical factor is causing noticeable deviation from the statistical correlation pattern but does not give unbiased estimates of compensation temperatures.

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Age of the lower flood basalts of the Ethiopian plateau

VARIOUS stages of continental fracturing and crustal separation are displayed in Ethiopia at present. Separation of the African and Arabian plates has taken place along the Red Sea and Gulf of Aden rifts and in several areas of Afar where axial volcanic ranges are composed of low-potassium oceanic tholeiites[1]. The onset of spreading was accompanied by deep faulting and intense fissural activity, with the eruption of very fluid flood basalts over Mesozoic sediments and Precambrian basement. The flood basalts outcrop on the plateaux flanking the rift system (Fig. 1).

The determination of radiometric ages from the flood-basalt succession is important in the reconstruction of the tectonic and magmatic events which preceded rifting. Following the dating of basal flows from four localities[2], 22 ages have been determined from flows higher in the sequence and from other localities, shown in Fig. 1 These results show the time range during which the lavas were erupted and they also provide a stratigraphic control on the validity of the basal ages K–Ar ages were measured on whole-rock samples and the new data are presented in Table 1, together with the brief petrographic descriptions. Errors quoted are at the 60% confidence limit and based on replicate analyses.

With the exception of one sample (30940) an excellent stratigraphic consistency is maintained throughout the profiles and the age ranges are shown in Fig. 2, which is a compilation of the new data reported here and those reported before[2–4]. The age of sample 30940 has been omitted from Fig. 2 because the other ages from that profile suggest that sample 30940 has suffered argon loss. In only one profile examined do the basalt ages exceed 30 Myr, that is the Wolkefit Pass profile in the Simien massif (36 Myr). This supports the conclusion[2] that the major part of the plateau lavas is much younger than previously thought. In a paper by Mohr[5] (Fig 1) a histogram of all other available age data also supports this conclusion, and it is interesting to note that Mohr has omitted the older ages of Megrue *et al* [6] due to geological inconsistencies, as well as omitting his previous data from the Blue Nile Gorge[7] which have been shown to be erroneous[2,3]. Although it cannot be stated definitely that any of the flows dated in this work represented the oldest volcanics of the province, some of the data disagree with the sequence produced by Zanettin *et al.* which extends to much older ages[8–10] Specific examples of these inconsistencies are in the Blue

Table 1 Sample data

Sample no.	Profile	Position in profile	%K	Volume ^{40}Ar radiation standard cm^{-3} g^{-1} × 10^{-9}	% ^{40}Ar radiation	Age (Myr)	Petrography
30406	Aisha	Upper flow	0.67	0.5375	69.3	19.8±0.7	Coarse olivine and pyroxene-phyric basalt with matrix of plagioclase, pale brown augite, and magnetite
30405		Lower flow (tilted traps)	1.11	0.8877	66.8	19.9±0.7	Fine-grained pilotaxitic basalt Sparse plagioclase phenocrysts in a quenched matrix of plagioclase, clinopyroxene and opaque ore
30412	Jijiga	Sheet in sandstones	0.38	0.3062	28.1	20.1±0.7	Medium-grained dolerite with clinopyroxene and plagioclase phenocrysts in matrix of similar composition plus olivine and opaques.
30414		Top flow	0.76	0.6911	39.8	22.6±0.7	Clusters of zoned plagioclase phenocrysts in fine-grained matrix, with approximately 8% brown isotropic glass
30437	Cassam	Top basic flow	1.14	0.4067	61.0	8.9±0.3	Fine aphyric basalt Plagioclase (An_{55}), augite and quenched matrix with dusty opaque ores
30444		130 m from top	1.11	0.3904	59.4	8.7±0.3	Euhedral plagioclase phenocrysts (An_{58}–An_{53}) in similar matrix as above with 5–10% isotropic glass
30446		150 m from top	0.88	0.3464	53.2	9.8±0.3	Similar to 30437 Intergranular matrix
30878	Maichew	140 m from base	0.92	0.9691	79.1	26.2±0.8	Approximately 12% pyroxene and olivine phenocrysts in an intergranular matrix of plagioclase, clinopyroxene, and opaque ore.
30875		110 m from base	0.97	1.0003	85.6	25.7±0.8	Pyroxene-phyric basalt with quenched matrix containing opaque ore laths. Approximately 10% glass.
30947	Adigrat	Dyke	0.28	0.1746	39.2	15.5±0.5	Olivine dolerite Slightly serpentinised olivines in ophitic matrix of plagioclase, opaque ore and pinkish augite
30905		Top flow (640 m)	0.92	0.6962	38.1	18.8±0.6	Dense black microporphyritic basalt with feldspar laths in a pilotaxitic matrix containing abundant opaque ore.
30908		480 m from base	0.92	0.7397	47.3	20.1±0.7	Aphyric basalt Pinkish-brown augite, rods of opaque ore and ragged feldspar laths.
30895		100 m from base	0.21	0.1879	39.2	22.4±0.7	Aphyric patchy texture of aligned feldspar laths in subophitic augite and greenish quench Sparse opaques.
30911	Garamullatta	610 m from base	1.27	1.0573	73.3	20.8±0.7	Fine-grained aphyric basalt. Plagioclase (An_{38}–An_{55}), pale brown augite and opaque ore. < 5% isotropic glass
30927		360 m from base	0.71	0.5895	61.7	20.7±0.7	Plagioclase and clinopyroxene phenocrysts in a cryptocrystalline quenched matrix.
30942		180 m from base	0.78	0.7597	62.8	24.3±0.7	Subophitic basalt Plagioclase laths, small olivines and euhedral opaque ore in clinopyroxene
30971	Mugher	300 m from base	1.13	0.8967	83.6	19.8±0.7	Sparsely porphyritic basalt Clinopyroxene phenocrysts, zoned at margins, in plagioclase–clinopyroxene–opaque ore matrix.
30967		260 m from base	1.00	0.8612	81.0	21.5±0.7	Clinopyroxene, olivine, and plagioclase phenocrysts in a pilotaxitic matrix of similar composition
30958		180 m from base	1.14	0.9932	81.4	21.6±0.7	Fairly coarse basalt Plagioclase (An_{67}), altered olivine, clinopyroxene and opaque ore in seriate texture
31000	Wolkefit Pass	1,150 m from base	1.04	0.9526	77.1	22.8±0.7	Approximately 40% large plagioclase laths in a matrix of pinkish-brown clinopyroxene, plagioclase and opaque ore 10% isotropic glass.
30999		1,030 m from base	0.46	0.4985	48.9	27.0±0.8	Clusters of plagioclase phenocrysts in similar matrix as below
30991		760 m from base	1.19	1.5130	23.6	31.6±0.9	Big-feldspar basalt Zoned plagioclase phenocrysts in quenched matrix of plagioclase brown augite and approximately 10% opaque ore
30988		300 m from base	0.47	0.6806	49.6	36.0±1.1	Feldspar-phyric basalt Approximately 15% plagioclase (An_{65}–An_{55}) in holocrystalline pilotaxitic matrix.

$\lambda_\varepsilon = 0.584 \times 10^{-10}$ yr^{-1}, $\lambda\beta = 4.72 \times 10^{-10}$ yr^{-1}, ^{40}K $= 1.19 \times 10^{-11}$ mol per mol K.

Nile and Mugher Gorge profiles. The Blue Nile volcanics, classified by Zanettin as Aiba Basalts (34–30 Myr), have been dated in Canberra giving ages between 27 and 23 Myr (ref 3). Mugher Gorge basalts, placed in the Ashangi (older than 34 Myr) and Aiba (34–30 Myr) groups by Zanettin *et al.*, have been dated in Leeds between 22 and 19 Myr (work reported here). Due to these obvious discrepancies the chronological sequence of Zanettin *et al* cannot be confirmed.

There is excellent agreement between the data from the south-eastern profiles (Jijiga and Garamullatta) and work by Morbidelli *et al.*[11] and Kunz *et al.*[12] from localities further west on the same plateau. All the data indicate a maximum age of approximately 25 Myr for the onset of widespread volcanism on the south-eastern plateau.

Figure 2 shows that there is a general decrease in the ages of the profiles from north to south, indicating a possible southward migration of the initial activity This idea of southward younging was first mentioned by Dainelli[13] and later by Mohr[14] and Gass[15], although they believed it began much earlier in the Eocene epoch. Zanettin *et al.*[10] have also demonstrated the migration of the volcanism towards the rift zone. This apparent progression towards the rift is caused by the gradual subsidence of the area during volcanic activity, resulting in the restriction of the flows to progressively narrower zones. A similar process has affected the Iceland flood basalts (I. L. Gibson, personal communication).

The Jijiga profile provides an interesting date from the intrasedimentary basalts (30412). From field relationships it is uncertain whether the basalts are flows within the sandstones, and therefore of Mesozoic age, or younger

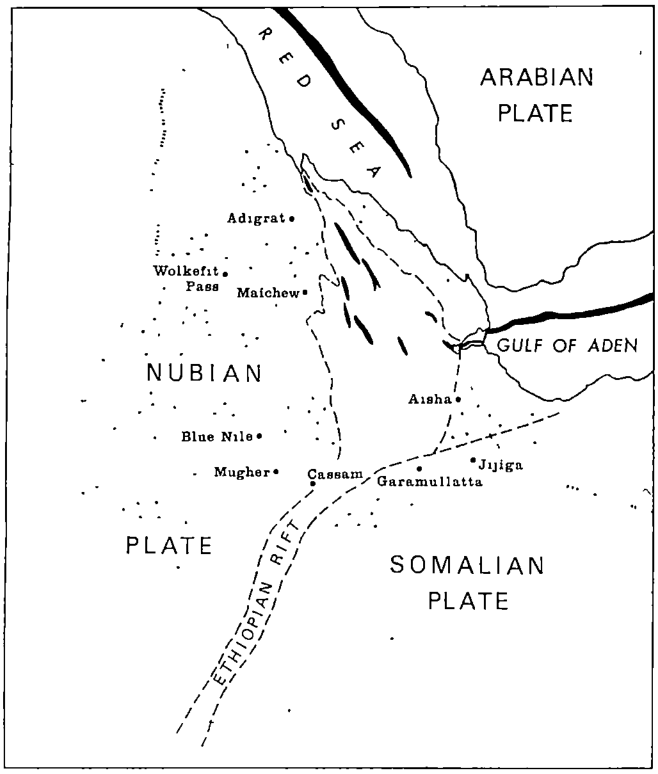
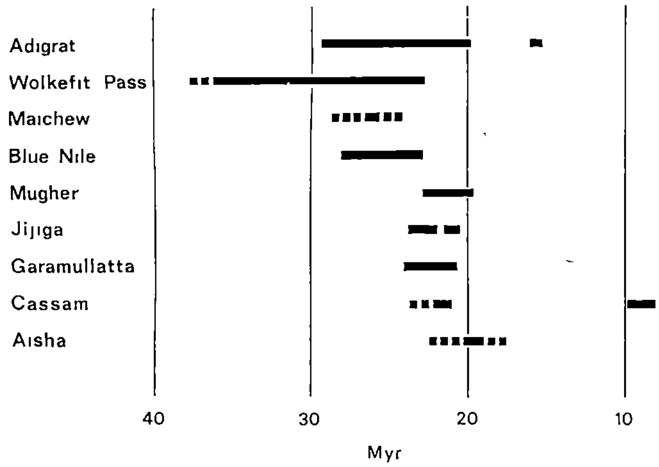


Fig. 1 The rift system in Ethiopia. Black areas, zones of generation of new oceanic crust. Stippled areas, Ethiopian plateaus Solid circles, profile localities.

intrusives. Mohr[14] considered these to be Mesozoic flows. The radiometric age of 20.1 Myr shows that they are intrusive sills emplaced during the profuse igneous activity at that time.

An important conclusion from all these new data is that there was widespread eruption of flood basalts over the plateau areas during the period 25–19 Myr ago (Fig. 2). It had been proposed[15] that the trap basalts represent a separate stage of volcanism which preceded the formation of the Afar depression. But according to Barberi *et al.*[16] the Afar depression began to form approximately 25 Myr ago, and there is therefore a large overlap of ages from the plateau and Afar regions. This shows that flood basalts were being erupted on the plateaux even during the formation of the depression, and that igneous activity was contemporaneous in the two regions for a long time. The volcano-tectonic sequence during the initial fragmentation of the continental plate in north-eastern Africa must be accommodated within a time range which is much shorter than in previous models[14,15].

Fig. 2 Age ranges obtained from the nine profiles. Broken lines indicate where the plateau basalts continue above or below the measured profile.



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The Marda Fault Zone, Ethiopia

The Marda Fault Zone in south-eastern Ethiopia was first recognised in the Marda Range near Jijigga (Fig. 1) and was called the Marda Hills line. The "linear NW–SE arrangement of basalt capped summits with basaltic plugs" and the associated Bouguer anomaly were considered indications of a major "volcanic-tectonic" lineament[1]. Subsequently the fault zone was described as a complex of NW–SE trending faults, down thrown to the NE and possibly extending 200 km into the Ogaden Basin[2]. Recent studies have indicated that the fault zone extends over 400 km beyond the Marda Range to the Belet Uen area in Somalia[3]. Moreover, a major fault zone trends south-east from Belet Uen to the Somalia coast and can be considered a further extension of the zone[4]. These indications of a zone of faulting from the southern margin of the Afar Depression south-east to the Indian Ocean define a length for the Marda Fault Zone of >900 km. The feature must therefore be recognised as a major structural element in the Horn of Africa. We here attempt to define the structure and age of the zone, and have made use of ERTS-1 (LANDSAT) Band 7 imagery, supplemented locally with colour composites, detailed photostudy between 7°50′N and 9°00′N, and potential methods surveys near Daghabur to supplement earlier geological and geophysical surveys.

The ERTS imagery clearly shows the outcropping volcanics and Hamanlei Formation sediments in the Marda Range, the linear valleys of the Fafan, Gerer and Wabi Shebelli rivers, and numerous linears (Fig. 1) between 10 and 100 km long, several of which near Jijigga have been proved to be wrench faults[3]. Numerous NE–SW linears are also seen, which in the southern Ogaden seem to offset the zone. Similar cross-faulting has been mapped on the ground near Jijigga[2]. Structural form lines determined from air photography consistently swing to the right near the zone (Fig. 2) suggesting dextral displacement on the faults.

South of Daghabur a positive Bouguer anomaly of ~40 $\times 10^{-5}$ m s^{-2} trends NE–SW on the eastern side of the Marda lineament and east–west on the western side (Fig. 3). Similar Bouguer anomalies in the zone near Shilavo and Abred are associated with significant basement structure[5], though the anomalies can only be adequately explained by assuming superposition of intrabasement and suprabasement density contrasts as in the El Nala Bouguer anomaly in Saudi Arabia[6]. Profile analysis of the Daghabur anomaly defines a residual anomaly of almost 5×10^{-5} m s^{-2}, which may indicate suprabasement structure. This is supported by a negative magnetic anomaly of $\sim10^{-7}$ T coincident with the gravity anomaly. The main anomaly may be interpreted in terms of crustal structure similar to the Bouguer gravity anomaly on the zone near Jijigga. In this case, major arching of the Moho discontinuity appears coincident with the Marda Fault zone[7].

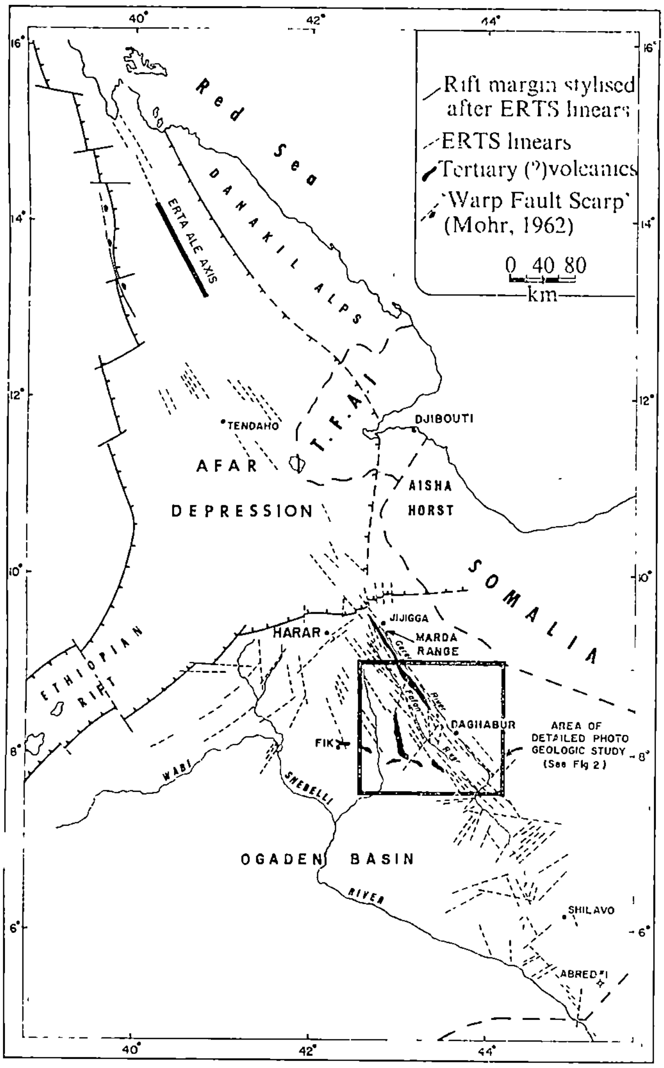


Fig. 1 Location map showing ERTS linears along the Marda Fault Zone Only linears on trend with the zone are shown in the Afar Depression and most linears on the southern Somalia plateau have been omitted for clarity. 'Tertiary Volcanics' refers only to the volcanics on the Plateau in the vicinity of the Marda Fault

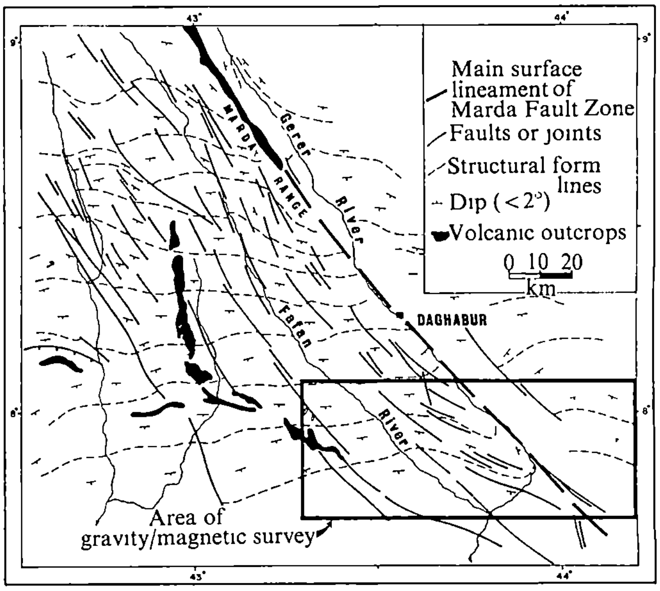


Fig. 2 Structural photogeology map of the Marda Fault Zone between 9°00′N and 7°40′N. This interpretation is part of an unpublished company report by Photogravity Inc., Houston

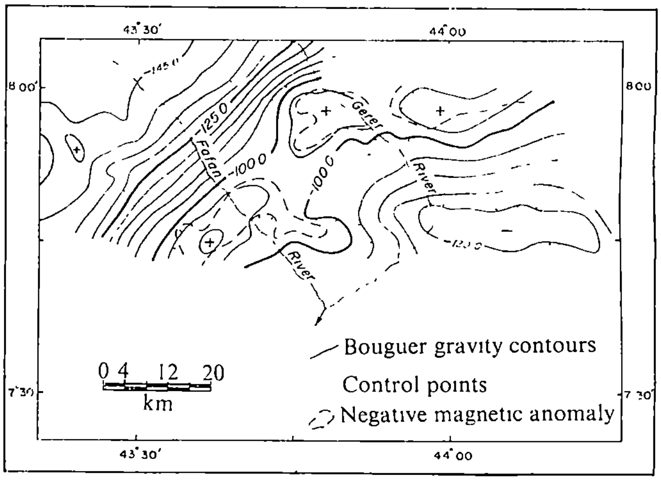
Black *et al.*[3] defined a Tertiary age for the zone, evidenced by the abrupt contact of Mesozoic and Tertiary outcropping units across the zone, the displacement of Mesozoic sediments by NW–SE faults near Jijigga and the volcanics of the Marda Range which locally overlie the Cretaceous Amba Aradam sediments[8]. Mesozoic activity on the zone is, however, evidenced by structural thinning of the lower Jurassic Hamanlei Series on the basement structures, confirmed by reflection seismic surveys, and the thinning of Upper Jurassic limestones west of Jijigga[9]. The large Bouguer anomalies on the zone and the basic change in character of the regional Bouguer field across the zone have been interpreted as indirect evidence of Precambrian tectonism[5]. Mohr[10] has noted that the NW–SE faulting near Tendaho, the alignment of Pleistocene volcanic centres on the Afar floor and the sharp reduction in the magnitude of the Afar margin structures east of the Marda Fault, point to the "continuing influence of the Marda line on Neogene tectonism of the southern margin of Afar despite the apparently extinct tectonism of the line itself" These indications of activity in the zone from Precambrian to Recent times qualify the proposal that the Marda Fault marks a zone of crustal weakness, continuing the Red Sea trend across the horn of Africa.

The fault zone has been projected via a line of epicentres into the Erta Ale volcanic axis, possibly as part of a major mid-Tertiary shear zone extending along the Red Sea[5]. Alternatively, the fault has been projected north-westwards across the Afar into the highlands of Eritrea where it corresponds with a zone of Precambrian faulting and a significant facies change in the Jurassic limestones[5].

Current detailed geological mapping of the zone southwards to 7°50′N and projected additional gravity and magnetic surveys will hopefully provide new insights into the nature and age of the fault zone and its regional significance.

I thank the management of Whitestone International Inc., and L L. & E International Inc., for permission to comment on results of the companies' current exploration

Fig. 3 Bouguer gravity map of a portion of the Marda Fault Zone between 7°30′N and 8°10′N.



programme. Special thanks are also due Dr J. Walsh who reviewed the text and to Robyn Purcell, Debrework Haile and Allemayehu Mesfin for their assistance.

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Prehistoric exploration at Hadar, Ethiopia

DURING the third International Afar Research Expedition, which led to the exciting find of the australopithecine skeleton known as "Lucy"[1], parallel investigations revealed the existence of two Early Stone Age (ESA) industries in the Hadar area. One comprises an Acheulian workshop (the Denen Dora Koma Acheulian), and the other, stratigraphically younger, constitutes a degenerate biface-flake industry.

The oldest known artefacts from the Hadar area (within the Afar Rift in Ethiopia) come from the Afaredo site and comprise a few basalt flakes from a silt within the upper part of the fluvio-lacustrine Hadar Formation. They have sharp edges and retain much of the cortex on their dorsal faces, besides one or two former flake scars The calcareous, in part tuffaceous, silt occurs in an 8-m section of silts and sands on the bank of the Afaredo River and contains a small channel of tuff about 0.50 m thick. The flakes were embedded in the top of the silt at the contact with fine sand, constituting an ancient weathered horizon with small lime pellets. The silt is stratigraphically younger than the hominid levels but older than the Acheulian horizon capping the Hadar Formation. The time interval separating the hominids from the flake-bearing silt horizon seems to be shorter than the interval between the flake horizon and the Acheulian. Pending more detailed work, these findings remain too tenuous to allow a precise interpretation.

Following last year's exploration[2] of the Denen Dora Valley, it has been possible to map more than 120 artefacts on the surface of Holocene terraces in one of the small western tributary gullies of the Denen Dora. The concentration of tools increased upstream.

As the tributaries cut into the Hadar deposits precipitous slopes are formed in the area of Denen Dora Koma (mountain) At the top, lying unconformably over brown clays of the Hadar Formation, is a series of fluviatile gravels, sands and silts, capped by a boulder bed about 60 m above the bed of the Denen Dora River. It is in this fluviatile series that the Acheulian horizon occurs, at the contact between a conglomerate and a cemented sand (Fig. 1). The section forms a vertical cliff about 10 m high, above the more gently sloping clays of the Hadar Formation.

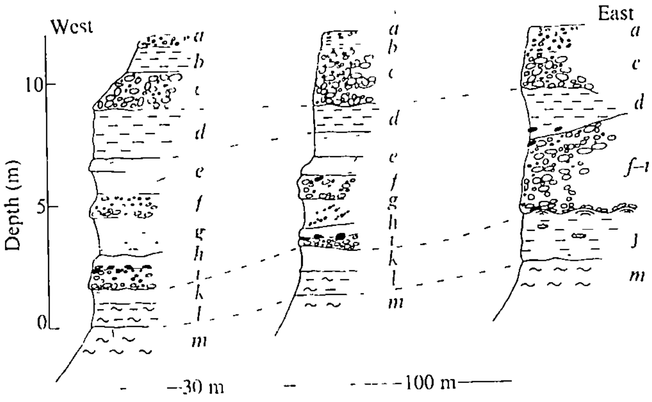

Fig. 1 East-west profile of site Hdr 56: *a*, residual gravel; *b*, thin layer of silt, disappearing to the east; *c*, boulder bed with occasional artefacts of the Hdr 10 type; *d*, brown, sandy silt; *e*, sands, partly cemented, partly loose; *f*, brown bouldery conglomerate, thinning out westwards; *g*, loose gravel and sand, disappearing eastwards; *h*, cemented sand with artefacts at the base; *i*, brown boulder conglomerate with artefacts at the top; *j*, grey, sandy silt with lime concretions and a lime crust at contacts with the boulder conglomerate; *k*, grey, loose sand with limonitic staining; *l*, grey, silty clay; *m*, brown clays of the Hadar Formation.

The horizon seems to be extremely rich, and the whole slope was found to be covered with bifaces. The artefacts are fresh and sharp. Small flakes and chips with equally sharp edges are also present amongst the bifaces. Most probably the site was formerly an Acheulian factory. All the artefacts along the Denen Dora are derived from this site by erosion of the escarpment.

The Denen Dora Koma industry was evidently an Acheulian biface industry of an advanced character. Tools were found from the surface sites Hdr 9, 22, 23, 24, 25, + up, along the Denen Dora and *in situ* from sites Hdr 50, 55, 56 at the Denen Dora Koma. The assemblage comprises 226 artefacts collected in 1974, and 29 artefacts found a year earlier; 46 further artefacts from the same site derive from another industry (Hdr 10). Preliminary analysis of collected material reveals the tool classes shown in Table 1. Basalt, trachytic ignimbrites and rhyolites were used as raw materials. The assemblage is homogeneous in type and shape. No cleavers have been found; symmetrical, elongate-ovate forms with rounded points were evidently preferred for hand axes. They were made from flat and split pebbles. Most of the tools retain cortex near the butt or at the upper face. A few minature forms with sizes of 7–10 cm have also been found.

Geological evidence indicates that the Denen Dora Koma Acheulian was situated at the edge of a flood plain. The

Table 1 Material from the Denen Dora Koma Acheulian

Site (Hdr No)	9*		22*		23*		24*	25	25 up	50	55	56	42	Total
Bifaces	5	4	15	1	2	2	1	20	39	16	1	54	8	168
Scrapers												2		2
Flakes	1	1	3					9	5	2	1	38	4	64
Cores	1				1			1	2	1		3	1	10
Modified pebbles								3	1			6	1	11
Total	7	5	18	1	3	2	1	33	47	19	2	103	14	255

*Collected during 1973–74; all others collected in 1974.

Table 2 Material from site Hdr 10

	Excavation				Surface					
	1973		1974		1973			1974		
	Tr. I	Tr. II	Tr. III	*in situ*	10–I, –II	–III	10–I, –II	–III	–IV	Total
Bifaces			9				1	1	4	15
Protobifaces		1	2	2	1	10	2			18
Choppers	1			1	2	4		2		10
Polyhedrons	1					1				2
Discoids						1				1
Modified pebbles			8			5				13
Flakes	2	2	31	4	7	5	3	7	2	63
Cortex flakes		1	9		1	3	1	2		17
Flake-like pieces						1				1
Cores		1	11	1	1	2				16
Total	4	5	70	8	12	32	7	12	6	156

stream deposited coarse gravel in the channel in swift water, and sands along the point bars, which were apparently occupied by man. The site became sealed by coarse fluvial gravel and later by over-bank deposits as the channel meandered further east. The site was probably occupied only temporarily, and the artefacts will have been quickly buried under frequent flood deposits. So far no associated bones have been found.

The Denen Dora Koma sequence is a local series of fluviatile beds which crop out only at sites Hdr 50, 55 and 56, sandwiched between the Hadar Formation and a boulder bed. Otherwise the Hadar Formation is overlain directly and uncomfortably by the boulder bed. A trench (3×9 m), dug into the boulder bed at site Hde 10-III (which last year yielded artefacts of an apparently pre-Acheulian industry), revealed 70 artefacts dispersed throughout the deposit at various random levels. They exhibit a crude technique of manufacture: cores are unprepared, and flakes in most cases retain some cortex at the dorsal face. No choppers like those from the surface have been found, though there are several modified pebbles. A few bifaces show Acheulian features but are crudely made, and others can only be described as proto-handaxes. Nonetheless, the few bifaces recovered from the excavation indicate a younger age than previously suspected, especially as the Denen Dora Koma Acheulian occurs in a stratigraphically older horizon beneath the boulder bed.

The tools in the assemblage found at site Hdr 10 (Table 2) are characterised by minimal workmanship. The industry seems to have produced tools which could be used for certain types of work, with a minimum of flaking. The industry does not look as if it was heterogeneous and does not seem to have been transported from different sources.

Several nearby localities have yielded artefacts of the same type, all derived from the boulder bed : sites Hdr 33, 36, 37, 38 and 39 are surface sites on the terraces along the Sidi Hakoma River; sites Hdr 46, 47, 48, 49 and 53 are all from the boulder bed in the vicinity of Hdr 10, a little to the north-west. Site Hdr 62 is from the boulder bed between the Sidi Hakoma and the Afaredo rivers.

The industry at site Hdr 10 can be called a degenerate biface-flake industry, operated by a different and somewhat later group of people than the makers of the Denen Dora Koma Acheulian. They neglected the Acheulian tradition, or imitated it crudely. A rather similar phenomenon can be seen at Olduvai in Bed IV, where a true Acheulian culture is apparently more or less contemporaneous with that of a developed Oldowan with degenerate bifaces (M D. Leakey, unpublished).

It is interesting that in the Hadar area evidence of a degenerate biface-flake industry exists in the boulder bed stratigraphically above the advanced Acheulian biface assemblage which occurs in the underlying fluviatile sands. Both are *in situ* and are in juxtaposition at site Hdr 56. The boulder bed, lying unconformably over the Hadar Formation, is therefore younger than previously assumed, correlating with Bed IV at Olduvai rather than with Bed II.

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Australopithecus, Homo erectus and the single species hypothesis

An enormous wealth of early hominid remains has been discovered over the past few years by expeditions within eastern Africa. Evidence has been presented for the existence over a considerable period of time of at least two contemporaneous hominid species[1]. Some of this evidence is compelling, but some less so for a variety of reasons such as the lack of association, fragmentary specimens, geological uncertainties, equivocal anatomical differences and suchlike. Many of these new specimens are of great antiquity and have led to suggestions that an early form of the genus *Homo* was contemporary with at least one species of *Australopithecus* The evidence presented here deals not with the earlier stages of human evolution, but with the unequivocal occurrence of *H. erectus* from the Koobi Fora Formation, east of Lake Turkana (formerly Lake Rudolf).

Among the variety of hypotheses put forward to accommodate the evidence in an evolutionary framework, the most explicit and directly simple is the single species hypothesis[2]. This hypothesis rests on the assumption that dependence upon tools was the primary hominid adaptation that enabled expansion into open country environments. In the clearest exposition of the hypothesis[3], basic hominid characteristics, including bipedal locomotion, reduced canines and delayed physical maturity are seen to have come about in response to more effective and greater dependence on culture. This assumption of a basic hominid cultural adaptation allied with the principle of competitive exclusion[4,5] leads to the conclusion that two or more hominid species would be extremely unlikely to exist sympatrically. Here we present decisive evidence that shows the existence of two contemporaneous hominid species in the Koobi Fora area.

The adult cranium KNM-ER 3733 (ref. 6) was found *in situ* in the upper member of the Koobi Fora Formation[7]. The sediments lie stratigraphically between the KBS and the Koobi Fora/BBS tuff complexes. The cranium consists of a complete calvaria and a great deal of the facial skeleton, including the nasal and zygomatic bones. The

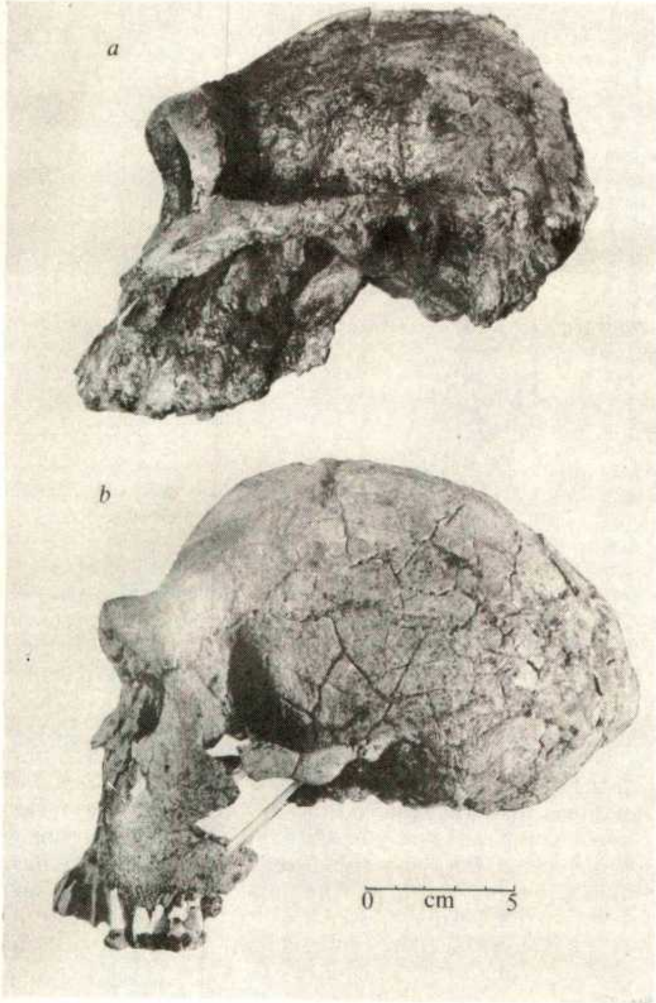

Fig. 1 Lateral aspect of KNM-ER 406 (*a*) and KNM-ER 3733 (*b*).

nearly complete alveoli of the anterior teeth and examples of the premolars and molars are preserved. The third molars were lost before fossilisation. Preparation has been limited so far to the external cranial surfaces and part of the brain case. An endocranial capacity is unknown, but by comparison is likely to be of the order of 800–900 ml. In all its features the cranium is strikingly like that of *H. erectus* from Peking[8]. Such orthodox anthropometric comparisons that can be made at present fall well within the range of the Peking specimens.

The cranium is large (glabella to inion/opisthocranion is 183 mm) with large projecting supraorbital tori and little postorbital constriction (minimum postorbital breadth 91 mm). There is a marked postglabellar sulcus and the frontal squama rises steeply from behind it to reach vertex at bregma. The skull decreases in height from bregma and the occipital bears a pronounced torus where the occipital and nuchal planes are sharply angled. The greatest breadth is low at the angular torus (biauricular breadth 132 mm). There are strong temporal lines that are 60 mm apart at their nearest point. As far as can be judged at present, the vault bones are thick (about 10.0 mm in mid-parietal). The temporal fossae are small. The facial skeleton is partly preserved and shows deep and wide zygomatic portions, longitudinally concave and laterally convex projecting nasals and wide, low piriform aperture. The incisive alveolar plane is short and wide and the large incisive alveoli are set almost in a straight line. There are strong canine juga. The palate is high and roughly square in outline. The facial skeleton is flexed under the calvaria and in the preliminary reconstruction is set at about the same angle as that reconstructed for a female *H. erectus* by Weidenreich[8]. The prosthion to nasion length is 87 mm. This is the best preserved single *H. erectus* cranium known, the facial skeleton being more complete and less distorted than that of 'Pithecanthropus VIII' (ref. 9).

Figures 1–3 show KNM-ER 3733 together with KNM-ER 406, another cranium discovered *in situ* in the Upper Member of the Koobi Fora Formation. This latter specimen has been described[10] in some detail and is clearly that of a robust *Australopithecus*. Other specimens of robust *Australopithecus* have been found in deposits of both the Upper and Lower members of the Koobi Fora Formation. Two *in situ* mandibles that must have been associated with this type of cranium are the most massive representatives of this species. KNM-ER 729 (ref. 1) was excavated in 1971 from the base of the Middle Tuff and KNM-ER 3230 (ref. 6) from the upper part of the BBS complex. Other, more incomplete, specimens of robust *Australopithecus* mandibles have come from the higher levels of the Formation between the Karari/Chari and Koobi Fora/BBS tuffs. The radioisotopic dating of these tuffs, in spite of a continuing controversy over one of them[11], is not an issue here. The contemporaneity of *Homo erectus* and a robust *Australopithecus* is now clearly established over the period during which the Upper Member of the Koobi Fora Formation was deposited. Using the time scale given by Fitch *et al.*[12], this would be from earlier than about 1.3 to earlier than 1.6 Myr ago.

The new data show that the simplest hypothesis concerning early human evolution is incorrect and that more complex models must be devised. The single species hypothesis has served a useful purpose in focusing attention on variability among the early hominids and also on the ecological consequences of hominid adaptations. Alternative concepts, especially those concerning niche divergence and

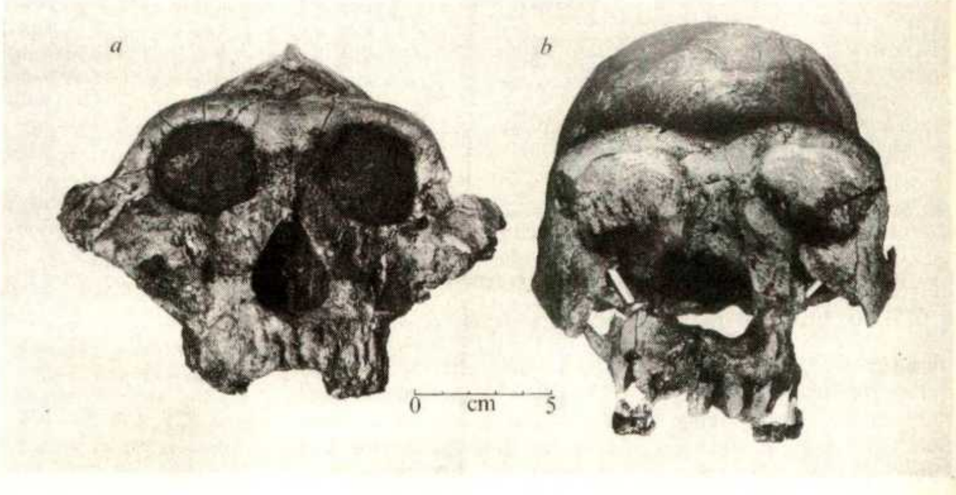

Fig. 2 Frontal aspect of KNM-ER 406 (*a*) and KNM-ER 3733 (*b*).

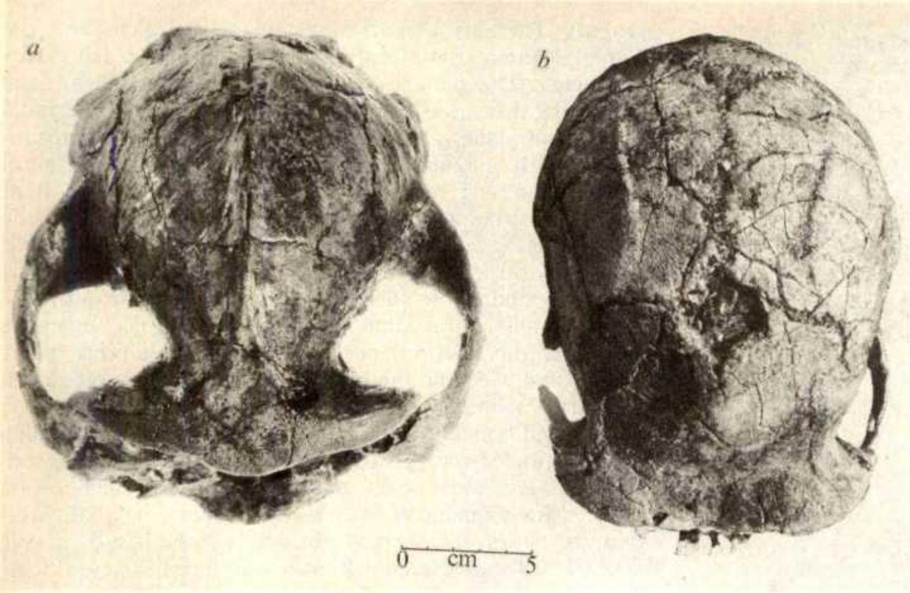

Fig. 3 Superior aspect of KNM-ER 406 (*a*) and KNM-ER 3733 (*b*).

sympatry, should now be formulated. We think that populations antecedent to *H. erectus* ones have been sampled in the Koobi Fora Formation (specimens include KNM-ER 1470, 1590 and 3732). The clear demonstration of at least two hominid species earlier in time should enable us to reconsider our approaches to the problems of earlier hominid evolution. We also think that there is no good evidence for the presence of *Australopithecus* outside Africa, and the finding of an apparently advanced *H. erectus* cranium at Koobi Fora provokes issues such as why *Australopithecus* is only an African form, the nature of its extinction and the apparent stability of some hominid morphologies over long periods of time.

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New hominid fossils from the Koobi Fora formation in Northern Kenya

A WELL preserved partial innominate KNM-ER 3228, and a relatively complete cranium KNM-ER 3733 provide further support for the presence of *Homo* during the Plio–Pleistocene in eastern Africa[1-5]. These hominid specimens and others (Table 1) were collected during the 1974 and 1975 field programme in the Koobi Fora Formation, lying to the east of Lake Turkana (formerly Lake Rudolf) in northern Kenya. (This area was previously known as East Rudolf and has been studied since 1968; ref. 6.) The material reported here has been referred to either the Upper or Lower Member of the Koobi Fora Formation. Recent work on the dating of tuffs by Curtis *et al.*[7] has raised important questions that will require time to resolve and may necessitate a complete re-examination of the reported chronology of the Koobi Fora succession and all other East African Plio–Pleistocene localities. Alternative dating methods such as fission track will be given close attention in the future and may offer, together with studies of certain taxa, useful indications about the reliability of isotopic age determinations.

The right innominate KNM-ER 3228 (Fig. 1) was discovered by Bw. Bernard Ngeneo in area 102 in the Lower Member. The specimen was found encrusted with a well

Table 1 Hominid remains from East Rudolf, 1974–75

KNM nos	Specimen detail	Area	Stratigraphic position (Member)
2592	Parietal fragment	6	Ileret
2593	Molar fragment	6	Ileret
2594	Proximal fragment of tibia and fragment of tibia shaft	6A	Ileret
2595	Cranial fragments	1A	Ileret
2596	Distal fragment of tibia	15	Upper
2597	Molar crown	15	Lower
2598	Occipital fragment	15	Lower
2599	Fragment of premolar crown	15	Lower
2600	Partial molar crown	130	Lower
2601	Molar	130	Lower
2602	Cranial fragments	117	Lower
2603	Tooth fragment	117	Lower
2604	Tooth fragment	117	Lower
2605	Tooth fragment	117	Lower
2606	Tooth fragment	117	Lower
2607	Tooth fragment	105	Upper
3228	Right innominate	10	Lower
3227	Mandible with both P_3 crowns	103	Upper
3230	Mandible with full dentition	131	Upper
3728	Shaft and neck of right femur	100	? Lower
3729	Body of left mandible, partial roots C–M_3	102	? Upper
3730	Weathered distal femur	102	? Upper
3731	Body of left mandible, partial roots I–M_3	105	Lower
3732	Partial cranium	105	Lower
3733	Cranium	104	Upper
3734	Body of left mandible, C–M_2, fragment M_3	105	Lower
3735	Weathered distal right humerus	116	Lower
3736	Proximal two-thirds of a radius	105	Upper

cemented matrix containing small pebbles and shells. The specimen seems to have eroded from a fluvial lens within a generally lacustrine section. The presence of similar pebbles and shells in this lens has been established.

The specimen is large, robust and from an adult male. The pubic and ischial rami are missing, having been broken away at the ilio-pectineal eminence at the inferior part of the tuberosity of the ischium. The acetabulum is large and it has been damaged along the pubic part of the rim. The position and orientation of the ischial tuberosity is similar to that observed in a sample of modern *H. sapiens,* as are a number of other morphological characteristics. Certain features such as the marked and thick acetabulo-cristal buttress are similar to those reported by Day[8] on the Olduvai innominate OH 28, and there are few resemblances to the material referred to as *Australopithecus*[9]. The apparent lateral flare of the ilium is accentuated by the acetabulo-cristal buttress and the marked concavity of the postero-lateral region of the iliac blade. Measurements are given in Table 2 and a detailed description will be presented elsewhere.

The cranial fragment KNM-ER 3732, discovered *in situ* but extensively weathered, includes the major part of the left orbit, the frontal region and the top of the vault, which is broken away near the squamous and lambdoidal sutures. The specimen is fractionally smaller, but strikingly similar to KNM-ER 1470 (refs 10 and 11) and thus provides a further example of this pattern of cranial morphology in the early hominids.

The cranium KNM-ER 3733 (ref. 12) was discovered by Bw. Ngeneo in area 104 in deposits of the Upper Member. The specimen was *in situ,* the supraorbital tori being just exposed on the surface. The maxillary and facial skeleton was fragmented and exposed within a distance of about 50 cm from the vault. Tooth crowns and an assortment of small fragments were recovered by sieving the area immediately below the skull on the slope. The cranium is large with only a slight postorbital constriction. The supraorbital tori are prominent, protruding anteriorly. The specimen is very similar to the *H. erectus* material from China (R. E. F. L. and A. C. Walker, unpublished) and is therefore assigned to *H. erectus.*

The archaeological research group which works under the direction of Glynn Isaac, recovered a hominid fossil from one of its trenches. The specimen, KNM-ER 3230 (Fig. 2) came from the site FxJj 20 (east) which was being excavated by J. W. K. Harris as a part of his intensive study of the archaeology of the Upper Member. The fossil is a mandible with a complete dentition (Table 3), but unfortunately the rami are not preserved. It is a typical example of the robust *Australopithecus* of which numerous specimens have previously been reported from East Africa[1–5,13–16]. The mandible was found by Bw. Kitibi Kimeu while consolidated brown silts were being dug away to uncover a major archaeological occupation floor which had been identified previously. It lay approximately 15 cm above the main artefact-bearing horizon, and there were a few scattered flakes at the same level (J. W. K. Harris, personal communication). The circumstances do not necessarily constitute evidence of a significant connection between the particular hominid and a stone tool industry, since there are discarded artefacts scattered throughout the Upper Member silts over very wide areas.

The left femur KNM-ER 3728 was discovered *in situ* by Bw. Maundu Muluila in Area 100. The specimen has suffered extensive abrasion on the proximal and distal ends with the condyles, lesser and greater trochanter and head being lost. The marked antero-posterior compression of the long neck compares well with femora that have been assigned to *Australopithecus*[17], but the long slender platymeric shaft is unexpected and provides intriguing evidence for the possible variation of the femur in this genus.

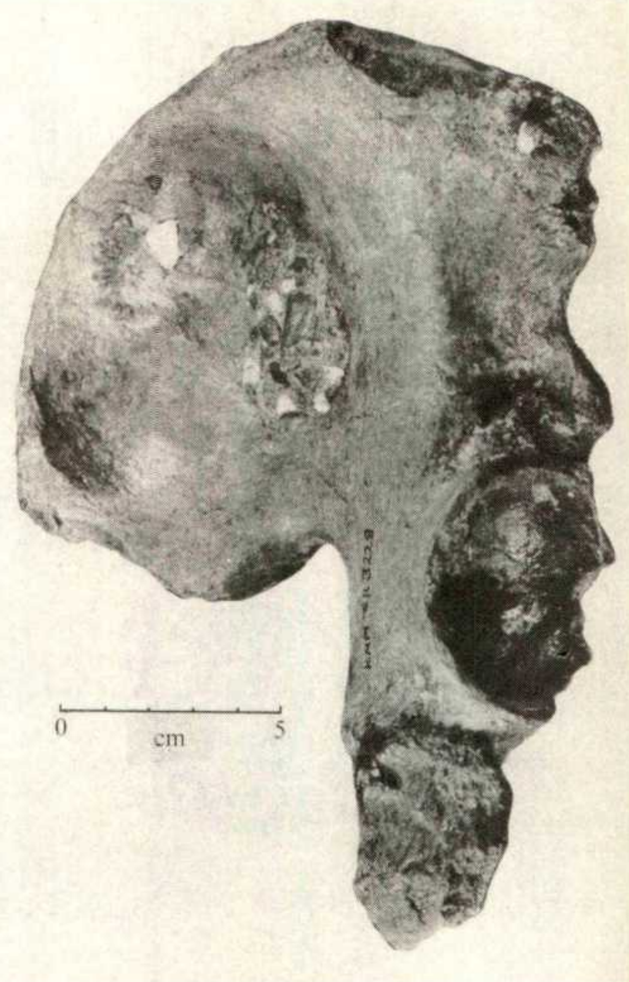

Fig. 1 Lateral aspect of the right innominate, KNM-ER 3228.

Among the new collection, the innominate is of particular interest and it can be clearly distinguished from the fossil innominates reported from Sterkfontein and Swartkrans.

Similarities to OH 28 from Olduvai Gorge and the essentially modern appearance of this fossil require that it be attributed to *Homo.* Robinson[18] proposed that the large acetabulum and the well developed acetabulo-cristal buttress were evidence that OH 28 was aberrant and that the Swartkrans fossils, such as SK 3155(b) be considered as examples of transitional *Homo* innominate morphology. He suggested a very late development of the modern hip in which the loading per unit area of articular surface is reduced. The new specimen requires that this argument be re-examined.

Similarly, the proposal by Wolpoff and Lovejoy[19] and others for a single pattern of pelvic morphology cannot be maintained. Although their observations on the mechanical

Table 2 Measurements (mm) of the innominate bone KNM-ER 3228

Anterior superior iliac spine to posterior superior iliac spine	166
Anterior superior iliac spine to posterior inferior iliac spine	147
Anterior superior iliac spine to deepest part of greater sciatic notch	115
Anterior superior iliac spine to centre of acetabulum	110
Posterior inferior iliac spine to superior margin of acetabulum	105
Diameter of acetabulum (supero-inferior)	57
Thickness of acetabulo-cristal buttress just above acetabulum	25

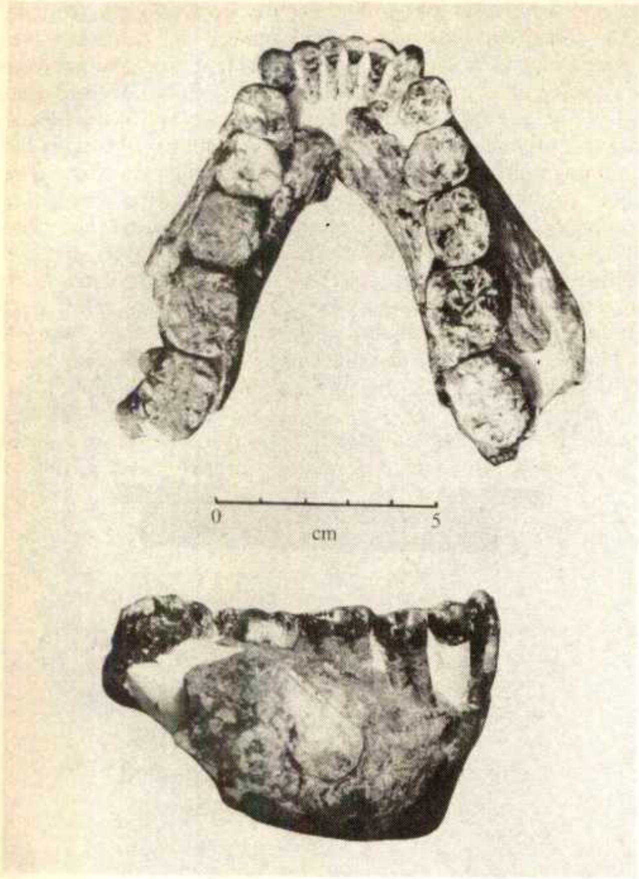

Fig. 2 Occlusal and right lateral aspects of the mandible, KNM-ER 3230.

issues involved in small brained, bipedal hominids may be correct, there is evidence for a second model which presumably reflects the mechanical requirements of a large brained, striding biped. The new innominate together with femora such as KNM-ER 1481 (refs 10 and 11) provide critical evidence for early modification of the hip joint in *Homo* and this adds weight to the argument for the late Pliocene separation of *Homo* and *Australopithecus*.

The East African fossil evidence for the Pliocene differentiation of *Homo* is still considered by some to be tenuous, but I am impressed by the cranial and postcranial data that are now available. There is an obvious need for more precise differential diagnoses. The latest attempt[19] is unsatisfactory because it is apparently based on an hypothetical model which is now invalidated by new empirical evidence. From early Pleistocene levels, and later, there does seem to be good evidence for the *Homo* lineage, and the principal specimens would include KNM-ER 1470 (ref. 10), 1590 (ref. 4), 1481 (ref. 10), the new innominate 3228 and the cranium KNM-ER 3733. The larger endocranial volume (upwards of 700 cm^3) and the large anterior dentition as well as the hip distinguish this group from other hominids.

Table 3 Measurements (mm) on KNM-ER 3230

	Left side		Right side	
	md	bl	md	bl
I_1	5.1	6.5	5.1	6.2
I_2	6.9	—	6.3	8.0
C	7.8	9.2	7.5	9.1
P_3	11.5	14.6	11.0	14.1
P_4	13.8	—	14.0	16.9
M_1	(17.0)	—	16.6	15.4
M_2	(20.0)	—	20.1	18.5
M_3	20.5	16.2	21.2	16.9

The question of species distinction within *Australopithecus* is problematical, but I am of the opinion that two contemporary species can be defined on the evidence now available. The most obvious is the robust form *A. boisei*[20] and this is well represented from late Pliocene horizons and continues until well into the Pleistocene (about 1 Myr). There may be some merit in treating this material as a subspecies of *A. robustus*. The evidence for a second species in East Africa is less convincing at present but there are specimens such as KNM-ER 1813 (ref. 4) that have the small endocranial volume (less than 550 cm^3) and generally gracile characteristics that seem to distinguish them from the robust species. One of the most distinctive features is the small size of the cheek teeth and small anterior teeth. Note also that at present, there is no convincing evidence for *Australopithecus* occurring earlier than examples of the *Homo* lineage. Certain specimens such as the Lothagam mandible have been attributed to *Australopithecus*[21], but the fragmentary nature of these specimens and the lack of diagnostic features would suggest that even generic attribution is not possible at the present time. Such specimens are more correctly referred to Hominidae indet.

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World's oldest animal traces

THE discovery of animal burrows in the basal beds of the main ore horizon on the Zambian Copperbelt provides evidence for the existence of metazoan life some 300 Myr older than the previously oldest known fauna, the late Proterozoic Ediacara fauna[1]. I describe here two kinds of burrow (referred to as types A and B pending more formal nomenclature), their geological setting, and age relationships.

Type A burrows are complex (Fig. 1); isolate forms are rare and multiple burrowing along narrow stringers (2–5 cm) is common. Complete bioturbation extending over several centimetres has been noticed at three horizons (Figs 2 and 3). The burrow walls are laminated parallel to the long axis of the burrow, and the infill exhibits a cuspate ('spreite') structure (Fig. 1), probably indicating active backfilling by the animal responsible. Branching of an irregular frequency is common, a simple Y shape predominating with no size change taking place. The burrows range from 3 to 8 mm in diameter and 3 to 10 cm in length, though

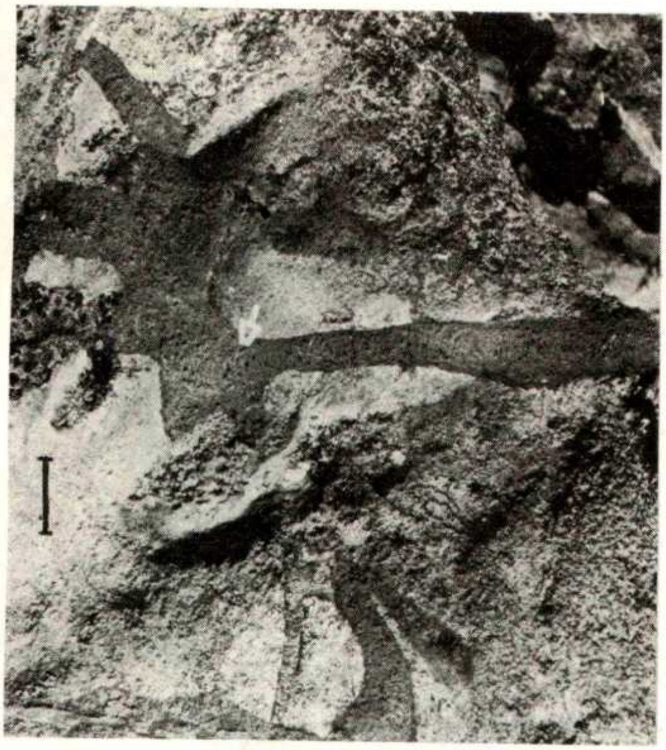
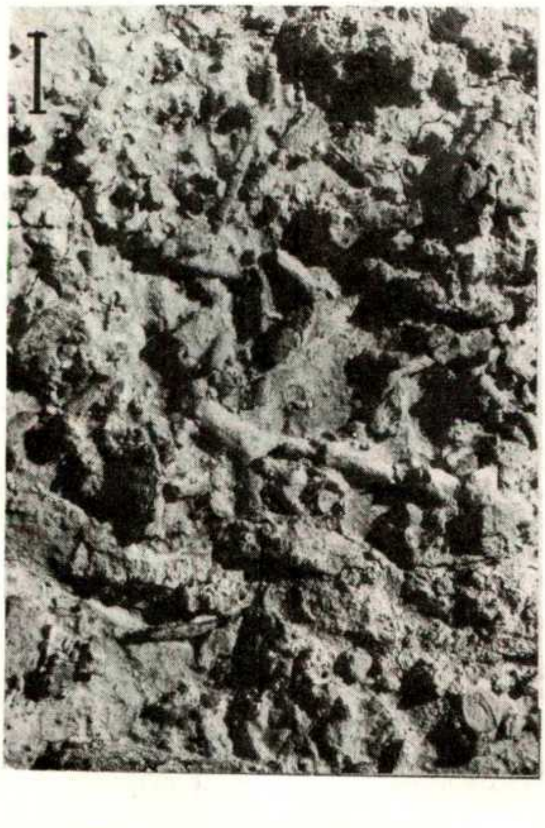


Fig. 1 Complex type A burrows. Infilling shows spreite structure (arrowed) and laminated walls. Colour contrast with the surrounding rock reflects the differential reaction to tropical weathering of the chemical and mineral changes induced during the passage of sediment through the animal's gut[6]. Mindola North Pit, Kitwe, Zambia. Scale bar, 1 cm.

burrows up to 20 cm long have been noted. No preferred orientation has been observed (Fig. 2) and the burrows lie horizontally with respect to the bedding. Occasional forms lying obliquely to the bedding have been observed, though these are rarely more than 1–2 cm long.

Fig. 2 Bioturbation showing type A burrows. Note lack of orientation. Mindola North Pit, Kitwe, Zambia. Scale bar, 1 cm.



Type B burrows are simple, usually isolate forms. (Fig. 4). The wall structures have been masked by secondary replacement and accretion, but it is evident that the burrows were lined and not actively filled. There is evidence of partial filling, attributable to diagenetic secondary minerals and to wall collapse which allowed sediment influx. Branching is common, varying from simple Y shape to dendritic. The frequency is irregular, and a size reduction after branching is apparent (Fig. 4). Diameters range from 2 to 10 mm with larger burrows frequently flattened to an ovate section. Continuous burrows, greater than 1 m in length have been observed (Fig. 4). The burrows lie horizontally relative to the bedding and no preferred orientation has been observed.

The sediments containing the burrows are the two basal units of the main ore horizon in the Roan Group of the Katangan Complex in Zambia (Table 1 and Fig. 3). The sediments represent the basal, littoral facies of a marine transgression which occurred after an extensive period of continentality[2]. During the early stages of the transgression the sea seems to have had intermittent connections with the main ocean, and the energy of the environment remained low until cycle 4 (Fig. 3). The unit overlying the burrowed horizons (top of cycle 2 on Fig. 3) displays evidence of highly saline conditions during deposition, and of reducing conditions in the sediment. Both of these factors would preclude the existence of an indigenous fauna, and it is notable that burrows are absent from this horizon.

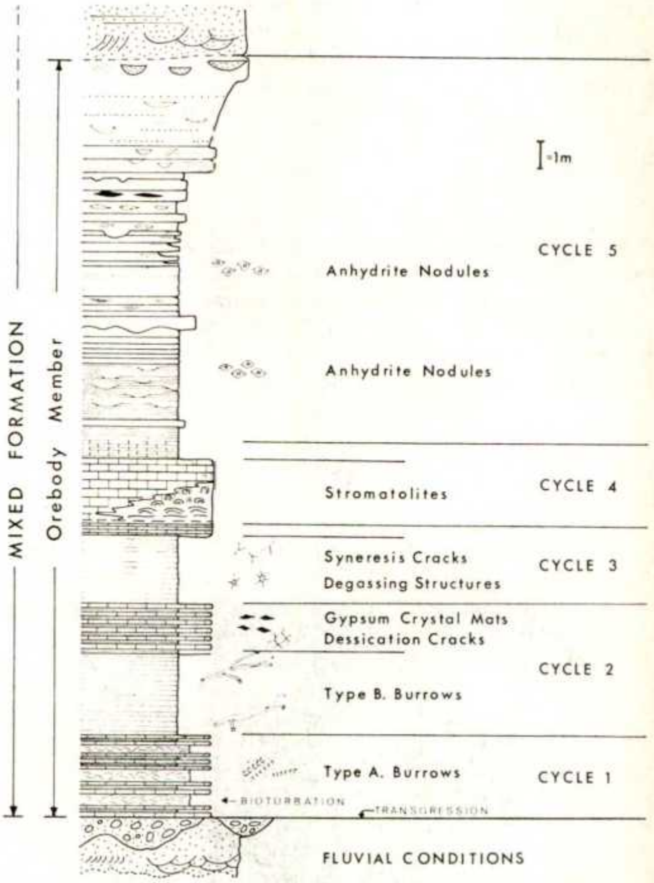


Fig. 3 Log of the containing sediment at the type locality. Each cycle consists of a deepening ('sub-tidal') and shallowing ('intertidal') facies. Cycle 1 is incomplete as only the 'intertidal' unit of the transgression is present. Mindola North Pit, Kitwe, Zambia.

A minimum age for the containing sediment is set by dates on syntectonic pegmatites from basal Roan Group rocks, of 888 ± 42 Myr BP and 840 ± 40 Myr BP (ref. 3). Combined lithostratigraphic, biostratigraphic (stromatolites), and chronostratigraphic studies have established[3] an age of

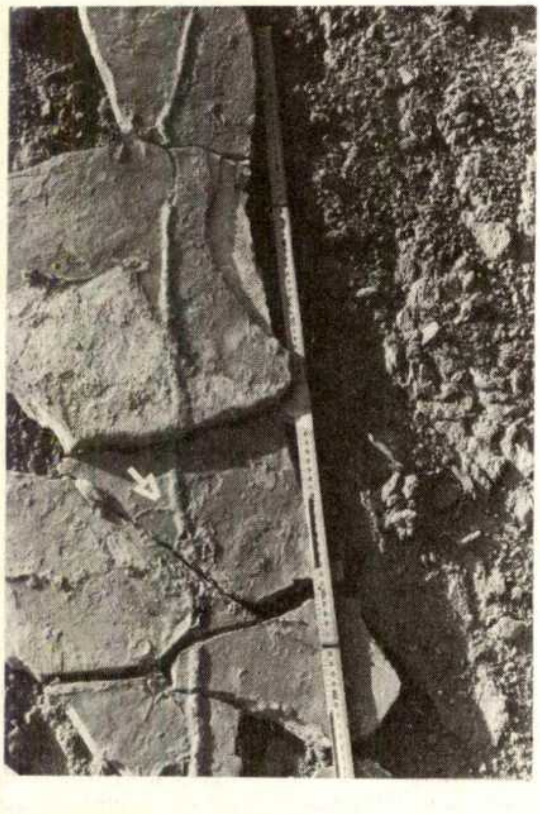
Table 1 Stratigraphy and approximate time range of the Katangan in Zambia and Zaire

COMPLEX	GROUP	FORMATION	Age (Myr)
KATANGAN	Kundelungu	Upper	620
		Middle (Petit Conglomerate)	680
		Lower	800
	Mwashia	(Grand Conglomerate)	950
	Roan	Dolomite	
		Mixed (Burrows)	1050
		Coarse Clastics	

1,000–1,050 Myr BP for the main ore horizon in the Roan Group of Southern Shaba in Zaire, and indicate that this horizon correlates with the main ore horizon in the Roan of Zambia; recognition of the depositional cycles depicted in Fig. 3 in the ore horizon of Komoto in Shaba (unpublished) confirms this. Thus an age of around 1,000 Myr for the burrows is reasonable.

The date of 1,000 Myr shows the burrows to be considerably older than the Ediacara fauna which has a time range of 600–700 Myr BP at 10 localities around the globe[1]. According to Glaessner[4] the oldest known evidence for animal life, the trail *Bunerichnus*[5], is found in the Brachina Formation some 1,500 m below the base of the Cambrian but 300 m above the uppermost glacials, the Eltina Group of the Adelaidean System in the Flinders Range of Southern Australia. The Marianoan Tillites of the Eltina equate with the Petit Conglomerate, uppermost of two tillites in the Katangan. The Sturtian Tillite of the lowermost Adelaidean glacials equates with the Grand Conglomerate, the lowermost of the Katangan Tillites (Table 1). The burrows reported here are at least 1,000 m below the Grand Conglomerate.

Fig. 4 Type B burrow. Note the simple form and size reduction on branching (arrowed). Mindola North Pit, Kitwe, Zambia. Scale in cm.



The question remains of why, after being at such an advanced stage of evolution in the basal Roan sediments, the animals did not continue to flourish, for the overlying sediments are devoid of animal fauna. The answer may be that the fauna was abandoned after encroaching on to the continent during the first transgression. The disappearance of burrows from the sedimentary column during the deposition of the highly saline beds at the top of cycle 2 on Fig. 3 could be a reflection of this.

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Explanation of large scale extinctions of lower vertebrates

A MAJOR area of speculation in palaeobiology is the search for the "causes" of massive cross-group extinctions such as that at the end of the Cretaceous. A controversial recent addition to the literature on the phenomenon of extinction itself is the work of Van Valen[1], who attempts to show that extinctions are stochastically constant over time for given groups of organisms. Lacking from Van Valen's study of group survivorship, however, is consideration of the history of diversification within each group. In an empirical study I have attempted to demonstrate features common to the diversifications of lower vertebrates and other organisms.

Figures 1 and 2 show the course of diversification of groups of lower vertebrates and certain invertebrates. For each group the numerical diversity (genera) at any given time is plotted as a percentage of maximum diversity observed for any similar time interval (the subdivision of the time axis, and thus the precision of the plots, varies according to the level of information available in compilations from the fossil record[2–4]). At the ordinal or subordinal level of traditional classifications of lower vertebrates a simple curve of diversity can be identified with a single major peak for each group; higher categories give multiple peaks. Although groups may persist in the fossil record for long times before the peak diversification, the curves for all groups are essentially similar between 25% points on the ascending and descending arms of the curve. The ascending slope of the curve approaches an exponential rather than a logistical growth pattern. The descending slope of each curve is usually quite symmetrical with the ascending curve, leaving only a short time span between 25% points on the curves. There are no major square-topped curves and the basic pattern is the same for groups diversifying at all times through the record. No group can be found that survives in the fossil record for significant lengths of time at or even near its peak diversity. Rapid extinction is thus a normal and immediate consequence of diversification for the groups shown.

This pattern is common to all groups of fishes, amphibians and reptiles for which adequate data are available[4]. It is also common to the following groups of invertebrates :

graptolites, cystoids, trilobites, nautiloids, Palaeozoic brachiopods, corals and ammonoids and possibly the ostracods. The following invertebrate groups (for which good data are available) do not show such rapid waxing and waning of diversity: bivalves, echinoids, gastropods. It is interesting that these last named seem relatively unaffected at the time of major extinctions of other groups[2].

The common shape of the plots of diversity is readily explicable in terms of existing theory[5,6] The basic characteristics defining a particular group are in evidence (that is, the group can be recognised) before the main diversification begins. The main diversification then occurs largely at lower taxonomic levels through production of large numbers of specialised lower taxa within a given adaptive zone. An analogy can be made between this situation and the phenomenon of "species packing" and consequent niche reduction[7] Regardless of the absolute number of taxa produced in each diversification, specialisation of taxa continues until a point at which normal environmental instabilities will make large numbers of taxa extinct. Hence the sharp decline in diversity after the peak. The specific components of the environmental instability may not be identifiable, but over geological time it is essentially a fundamental property of increased specialisation of taxa that some instability or other will cause extinction. Once diversification is entrained therefore (a process recognisable in hindsight as represented by approximately the 25% point on the ascending scale of the diversity curve) the rest of the process of diversification and extinction is predictable.

In addition to the shape of the "group diversity curves" the plots in Figs 1 and 2 show that the peaks in diversity of disparate groups tend to be coincident in time, rather than dispersed randomly throughout the record. This distribution of peaks seems to show a 62-Myr periodicity with less consistent 31-Myr intervals[4]

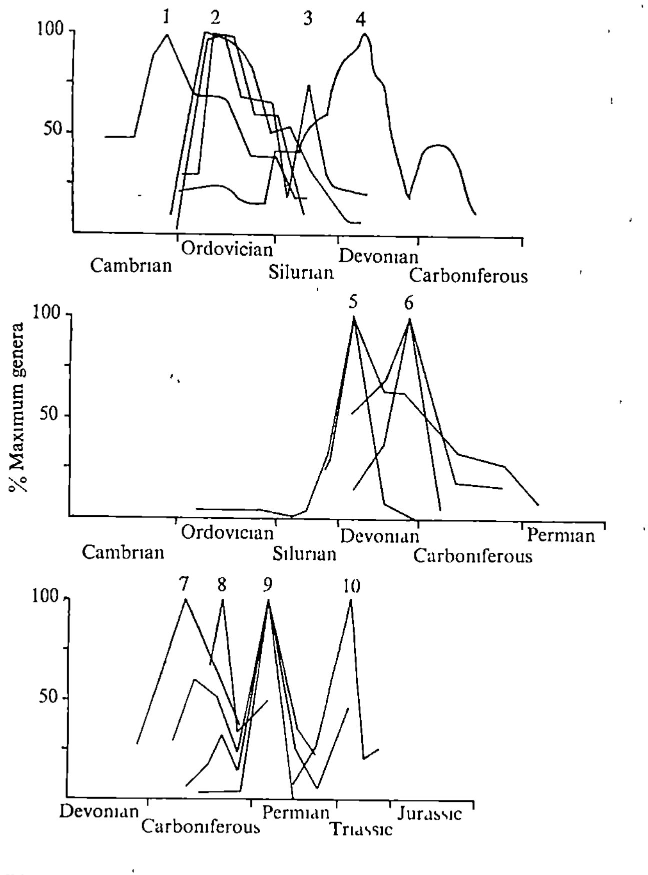

Fig. 1 Group diversity curves for the Palaeozoic The peak diversities of different groups are identified by number: 1, trilobites; 2, cystoids, graptolites, nautiloids, 3, nautiloids, 4, brachiopods; 5, agnathan and acanthodian fishes; 6, placoderm and sarcopterygian fishes; 7, chondrostean fishes and microsaur amphibians, 8, nectridean amphibians; 9, rhachitome and anthracosaur amphibians, 10, stereospondyl amphibians.

Comparison of Figs 1 and 2 shows that there is nothing different in the course of diversification and eventual extinction between groups going extinct in the Upper Devonian or Upper Cretaceous and any other groups. The major feature of the Permian is the lack of large numbers of groups diversifying in the Middle and Upper intervals. Permian vertebrates follow the common pattern. The Upper Devonian and Upper Cretaceous are remarkable, however, for the large number of groups diversifying more or less in synchrony.

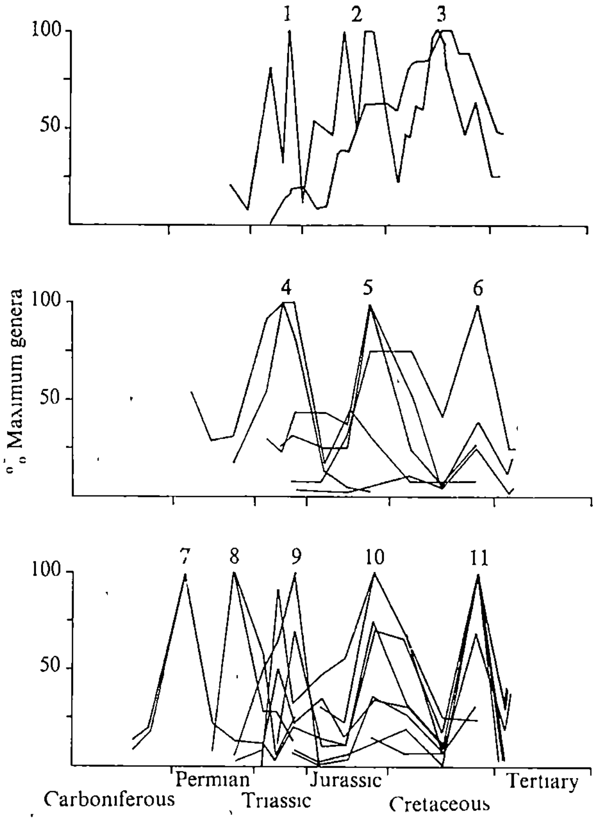

Fig. 2 Group diversity curves for the late Palaeozoic and Mesozoic The peak diversities of different groups are identified by number: 1, ammonoids; 2, ammonoids; 3, ammonoids and corals; 4, palaeonisciform and semionotiform fishes; 5, amiiform and pholidophoriform fishes; 6, pycnodont and teleost fishes; 7, cotylosaur and pelycosaur reptiles; 8, cotylosaur and eosuchian reptiles, 9, ichthyosaur, thecodont and saurischian reptiles; 10, pterosaur, ichthyosaur, saurischian and crocodilian reptiles; 11. chelonian, crocodilian, saurischian, ornithischian, sauropterygian and squamatan reptiles

If it is true that rapid extinction is inevitable we can refocus the search for causes. The important question about late Devonian and Cretaceous times (at least for the groups discussed here) is not why extinction occurred, but why so many groups diversified in synchrony at certain times in the Phanerozoic. It is necessary to find the major features of the physical and biological world common to these groups during their diversifications[8–11], rather than to find the signs of those minor perturbations that hasten their extinctions. Permo-Triassic extinctions of many invertebrate groups, however, still present a separate problem because there are no surges in diversity immediately preceding extinction

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Visual attention in split-brain monkeys

How the separated hemispheres of a split-brain animal avoid conflict with each other remains uncertain. In some circumstances both hemispheres seem to be able to attend to separate stimuli simultaneously[1,2]. Yet, when a split-brain animal has received separate training in each hemisphere on visual discrimination tasks which require opposite solutions, and is then placed in a situation where either hemisphere could respond, conflict does not occur. Instead, one hemisphere dominates behaviour for a variable time, then the other assumes control[3]. Independent variation of degree of attention in each hemisphere has been hypothesised to account for this[4]. We have sought electroencephalographic evidence of such hemispheric independence of visual attention in the split-brain animal, since a change in the power spectrum of the EEG accompanies increased visual attention —that is, an increase in θ (4–7 Hz) and β (13–25 Hz) frequency power, and a decrease in α (8–12 Hz) frequency power[5]. Power spectrum analysis has given evidence of alternate dominance of visual attention in the separated hemispheres. This finding has implications for the absence of hemispheric conflict in such animals, and the development of hemispheric specialisation in man.

Our subjects were three rhesus monkeys, which underwent surgical section of the telencephalic and tectal commissures, the optic chiasma, and in one case, the massa intermedia. During recording sessions the animals were seated in a restraint chair, allowed to gaze about them, but not required to make operant responses. Electrocorticograms from each occipito–parietal area were recorded on f m. tape, and later computer analysed to give average power spectra over successive 2-min epochs. Variations in the power spectra of each hemisphere were compared with those of its partner.

Usually the power spectra from each hemisphere covaried with the animals' general state of arousal and in those conditions the difference between successive 2-min spectra had a strong tendency to be closely similar in the two hemispheres. A relative increase in power in the θ and β bands was associated with a fall in the α band. On some occasions, however, the difference spectra in the two hemispheres would become almost mirror images suggesting a shift in the dominance of attention from both to one or other of the hemispheres. To determine whether these spectral shifts, which seemed to occur spontaneously, were related to the degree of visual processing in each hemisphere, we then checked the effect of covering each eye of the subject alternately, thus restricting visual input to one hemisphere at a time. Now difference spectra that were mirror images could invariably be produced by changing the cover from one eye to the other every 2 min (Fig. 1).

These observations suggest independent variations in the degree of visual attention in each hemisphere of the split-brain monkey. It seems that an increase in visual attention in one hemisphere may occur at the same time as a decrease in attention in the other hemisphere. Such a phenomenon perhaps involves a "switching" mechanism located at the midbrain level, or below, since in one case the midline bisection included the massa intermedia, as well as the tectal and telencephalic commissures, optic chiasma and associated supraoptic commissures.

A mechanism of this type would permit one hemisphere or the other to dominate behaviour in the presence of stimuli which might otherwise result in interhemispheric conflict. The factors determining which hemisphere achieves dominance at any given time remain unclear.

In the special case of split-brain humans, asymmetries of the EEG between hemispheres during performance of verbal or spatial tasks have been described[6]. A neural mechanism controlling hemispheric dominance of attention could be an important influence leading to hemispheric specialisation in man.

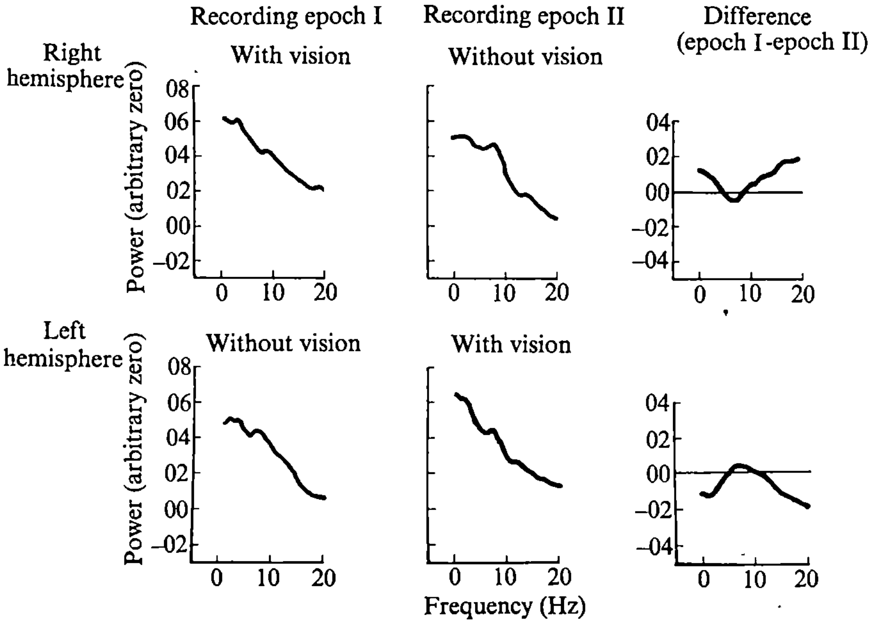

Fig. 1 The effect on the EEG power spectra of changing the cover from one eye to the other every 2 min. Difference spectra for the two hemispheres are mirror images, a relative increase in power in θ (< 7 Hz) and β (> 13 Hz) is associated with a fall in α (8–12 Hz) for the hemisphere visually attending. These results represent the average from three split-brain rhesus monkeys.

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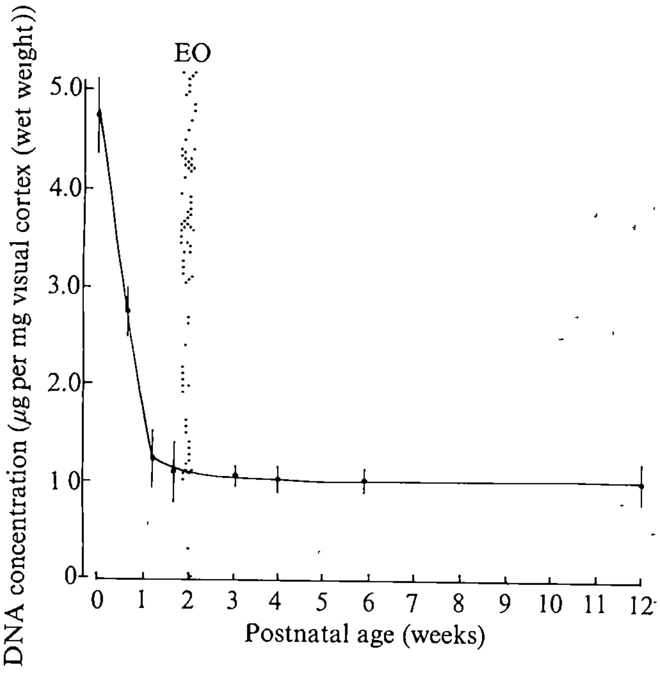
Tubulin synthesis in developing rat visual cortex

IN some species, restriction of sensory experience during early postnatal life can affect functional connectivity within the brain[1]. Yet, in these circumstances, little is known of the changes that occur to the internal organisation of brain cells It is also significant that certain membrane components of brain cells are continuously turning over and some of these are responsible for imparting to the membrane and cells their specialised functions[2] Thus plastic changes may sometimes result from a local alteration in the number or nature of molecules available for incorporation into the membrane, and it then becomes relevant to consider those systems which regulate the differential distribution of materials needed for maintenance and growth of cellular processes and synaptic junctions. Consequently, both here and in previous papers[3,4] we consider the possibility that the turnover of microtubular protein (tubulin) may be involved in some of the plastic changes in the brain that result from learning or early experience We report here significant changes in tubulin synthesis that are correlated with eye opening and the duration of a critical period.

Norwegian black hooded rats were reared under normal fluorescent lighting with a 12-h light/dark cycle (background illumination was approximately $100\ \mathrm{lm\ m^{-2}}$). At various times after birth the rats were killed and a plug of tissue removed from area 17 of the visual cortex in each hemisphere. Area 17 was located using the maps of Adams and Forrester[5] and Kreig[6] Plugs of diameter 2.0 mm and weight 6.0 ± 1.5 mg were removed from each hemisphere in animals up to 1 week old. Plugs of diameter 2.5 mm and weight 10.0 ± 2.0 mg were removed from animals aged 1 to 2 weeks, and plugs of diameter 3.5 mm and weight 15.0 ± 2.5 mg were removed from animals more than 2 weeks old. Care was taken to remove the underlying white matter.

Fig. 1 DNA concentration (determined by diphenylamine method) per mg wet weight visual cortex, as a function of postnatal age. EO, Eye opening.



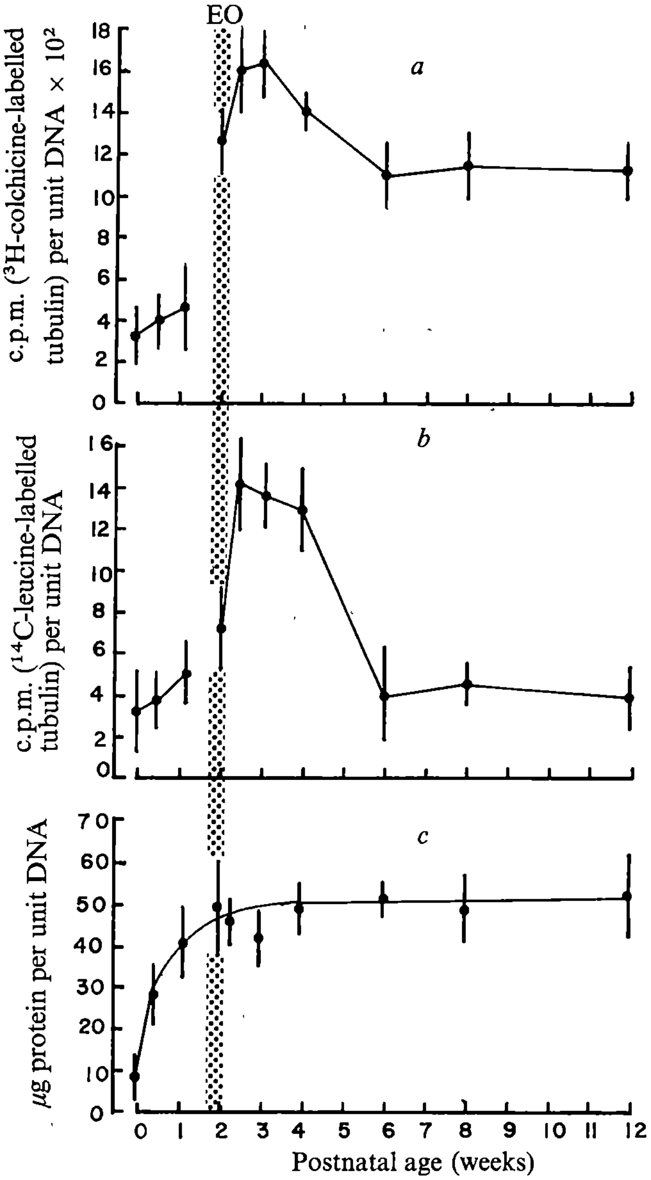


Fig. 2 *a*, Concentration of soluble tubulin in visual cortex. Ordinate shows c.p.m. ^{3}H-colchicine (Radiochemical Centre, Amersham) bound to tubulin that has been precipitated with vinblastine sulphate (Ely Lilly & Co Ltd). EO, Eye opening. *b*, Rate of soluble tubulin synthesis in visual cortex. Ordinate shows counts per minute ^{14}C-labelled L-leucine (Radiochemical Centre, Amersham) in tubulin that has been precipitated with vinblastine sulphate. *c*, Protein concentration (determined by Folin–Lowry method) per (DNA per mg wet weight visual cortex) Each point represents the mean value of at least four determinations Bars indicate standard deviations

Both the concentration of soluble tubulin and its rate of synthesis were determined by a double labelling technique adapted from a method described by Feit *et al.*[7]. Tritiated colchicine (200 mCi mmol^{-1}) was used to assay tubulin and ^{14}C-labelled L-leucine (348 mCi mmol^{-1}) injected into the brain ventricle 2 h before death was used to study rate of tubulin synthesis at different times postnatally. Because cortical cells increase their volume significantly in early postnatal life we normalised our results with respect to the amount of DNA per unit wet weight of visual cortex. Although cells may change their volume, the amount of DNA remains constant per cell, consequently, the amount of DNA per unit wet weight of cortex is proportional to the number of cells (per unit wet weight of cortical tissue).

Figure 1 shows how DNA per unit wet weight of visual cortex varies as a function of postnatal age. The value declines rapidly from birth until a few days before eye-opening when the curve reaches a level which then remains constant. The decrease in DNA per unit wet weight visual cortex in early postnatal life is probably due to the fact that cells are increasing their volume and cell processes are proliferating, so that there are fewer cells per unit volume of cortex.

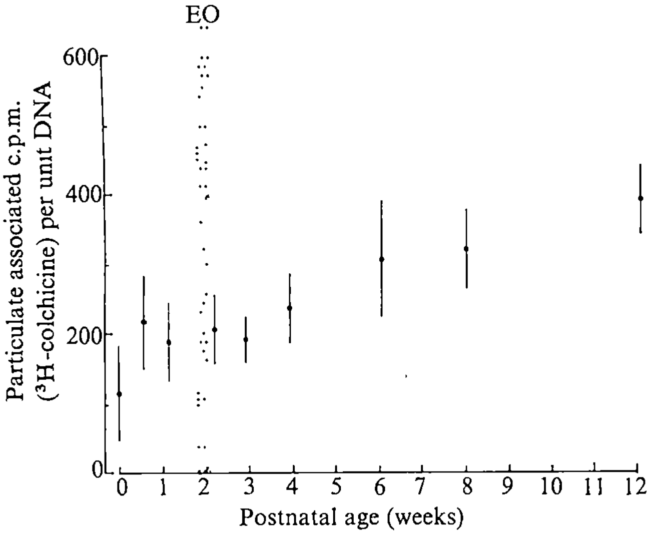


Fig. 3 Ordinate shows c.p.m. ^{3}H-colchicine bound to tubulin in particulate fraction.

Figure 2*a* shows how the concentration of soluble tubulin in the visual cortex varies with postnatal age. There is a marked rise in tubulin concentration at eye opening, and then a subsequent decline to a lower level which is maintained. This graph, however, not only represents tubulin that has been synthesised locally, but also includes tubulin that may have been synthesised elsewhere in the brain and 'imported' by cell processes and nerve fibres growing into the visual cortex from other brain regions A more accurate picture of the changes in soluble tubulin synthesis that occur locally is given by Fig 2*b*. This shows how the rate of synthesis of soluble tubulin varies with postnatal age. More especially, it indicates the amount of soluble tubulin that has been synthesised locally within the period of 2 h. As this is a relatively short period of time, it is unlikely that a great deal of newly synthesised tubulin will have been imported into our samples of visual cortex from elsewhere in the brain.

Following eye opening, both curves (Fig 2*a* and *b*), representing tubulin concentration and rate, respectively, rise dramatically. If we consider the rate of synthesis, we see that the curve drops again by about the sixth week to approximately the level it had attained at birth, and furthermore, the rate remains constant and maintains this level thereafter into adult life.

Figure 2*c* shows, for comparison, how the concentration of total soluble protein in the visual cortex varies with postnatal age. The curve is quite different from that for soluble tubulin, although clearly tubulin contributes to this curve Our results so far refer to soluble tubulin. Figure 3 shows the concentration of membrane-bound tubulin, that is, that found in the particulate fraction after high speed centrifugation, and again we find no obvious similarity to our previous curves for soluble tubulin.

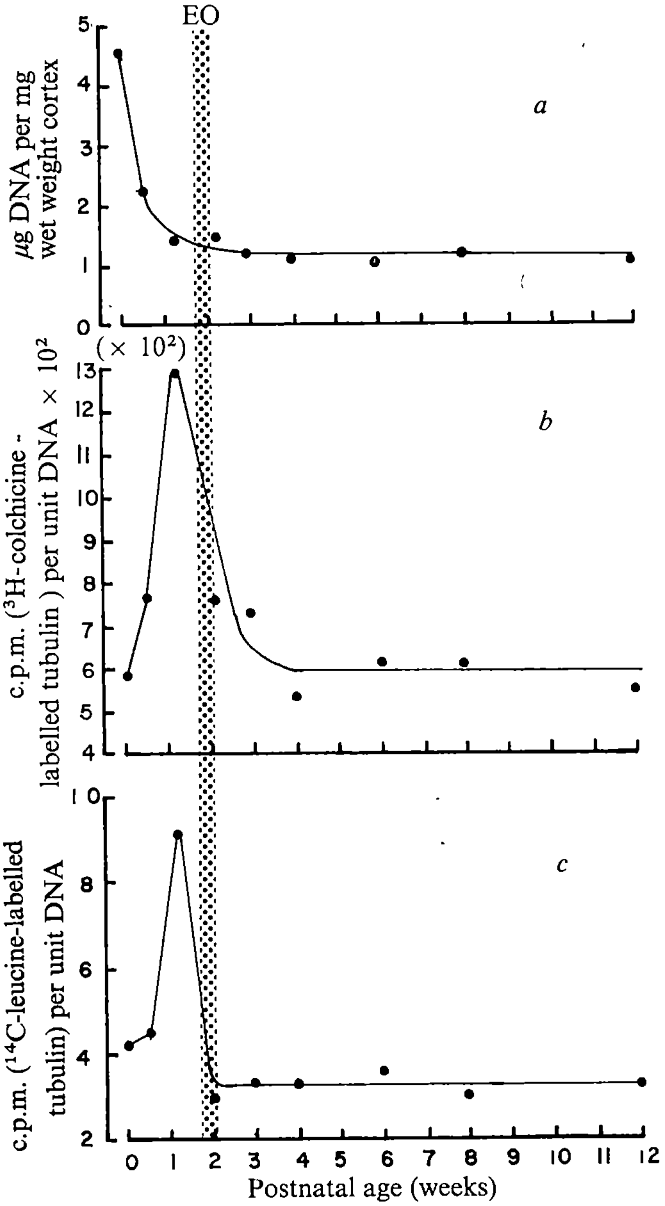


Fig. 4 *a*, DNA per mg wet weight cerebral cortex. *b*, Concentration soluble tubulin cerebral cortex. *c*, Rate of synthesis soluble tubulin cerebral cortex.

An important control is whether the correlation between the change in tubulin synthesis and eye opening is region-specific and restricted to the visual cortex, or whether it is also a characteristic of the whole brain. Figure 4 shows the corresponding measurements of concentration and rate of synthesis of soluble tubulin for the whole cerebral cortex. Again it is clear that the curves show no obvious correlation with eye opening and are similar to results obtained by previous workers for whole brain[8].

What functional significance may we attribute to this pattern of tubulin synthesis in the visual cortex? First, although the behavioural and electrophysiological evidence for the existence of a 'critical period' in rat visual cortex is meagre, we wish nevertheless to suggest that the peak in tubulin synthesis, which extends from eye opening until about the sixth week after birth may be correlated with a transient 'critical period' of increased plasticity and susceptibility to visual deprivation. Indeed, our 'biochemical estimate' of what we believe to be a 'critical period' in rat visual cortex, agrees broadly with the behavioural data of Walk and Walters[9] who have carried out experiments using the 'visual cliff' to examine the effects of dark rearing on the development of depth perception in the rat. It also agrees with the more recent behavioural data of Tees[10].

From a physiological standpoint, the consequence, to the cell, of having a sudden and large increase in the size of its available pool of soluble tubulin may be to enable the cell to maintain temporarily an uncommonly large number of synapses and cytoplasmic processes during the critical period. When the level of tubulin drops there may be a corresponding reduction in the number of microtubules since their subunit 'tubulin' only has a half life of about 4 d. Likewise, many cellular processes and connections may regress because there are insufficient microtubules to transport and keep them supplied with the essential materials needed for their maintenance.

At present, we are not yet able to affirm a causal relationship between eye opening and the sudden rise in tubulin synthesis. The answer here must await the results of experiments with dark-reared rats which are now in progress. Also, the possibility that genes controlling tubulin synthesis may be switched on by a sudden increase in neuroelectric activity, brought about by eye opening, is another hypothesis for which we hope soon to have an answer. Indeed, several examples are already known where neuroelectric activity at synaptic junctions affect gene action[11,12]; for example, in the autonomic nervous system, Thoenen[12] has shown that adrenal tyrosine hydroxylase activity may be increased by nervous activity, and his experiments provide evidence that the triggering of an operon regulating synthesis of noradrenaline may be brought about by neuroelectric activity. Clearly then, if during the 'critical period' in the visual cortex, major changes in macromolecular, and in particular, 'tubulin' synthesis are triggered by neuroelectric activity, this could in part account for the alteration in neuronal morphology and functional organisation that results from restriction of visual experience in early life. Answers to these questions must, however, await further experiments

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In vivo evidence for a circadian rhythm in membranes of *Gonyaulax*

IT has been suggested that rhythmic oscillations in membranes are the underlying mechanism for endogenous circadian rhythms[1,2] To support this suggestion, periodic oscillations in membranes of organisms displaying circadian rhythmicity must first be demonstrated. Rhythmic oscillations associated with membranes have been observed in the spontaneous firing of the optic nerve of *Aplysia*[3] and in the transmembrane potential of the pulvini of *Samanea*[4]. It is not clear if these membrane-associated rhythms are a function of specialised cells, or are basic phenomena of circadian rhythms. The only membrane-associated rhythm described in a unicellular organism so far is that of a circadian rhythm in particle distribution of one of the membranes of *Gonyaulax polyedra*[5]. We have now investigated the rhythmic changes in the membrane potential of *G. polyedra*. We used the method of Hoffman and Laris[6], in which fluorescent cyanine dyes are used to monitor the membrane potential *in vivo* of cells not amenable to the insertion of microelectrodes, in this case because of their small size and lack of a central vacuole. Our results suggest a temporal reorganisation of one of the membranes of *Gonyaulax* with circadian time.

G. polyedra 70A, a clonal strain isolated in 1970 by one of us (B.M.S.), was maintained in modified f medium (f/2) (ref.7), on an alternating 12-h light–dark schedule (1,500 μW cm^{-2}, 21 °C). Cultures were transferred to continuous light (300 μW cm^{-2}, 21 °C) at the beginning of the light period (0 circadian time, CT). At approximately 6-h intervals thereafter, samples of cells were collected by gentle centrifugation, washed three times with 10 volumes of 0.5 M NaCl, 1 mM Tris(hydroxymethylaminomethane)-HCl, *p*H 8.1, and finally suspended in 1 ml of the same solution. Next, 0.2 ml of the suspension containing $2\,5\times10^4$ cells was added to 2 ml of 100 mM NaCl, *p*H 8 1 (K$^+$-free medium) After equilibration for 15 min with stirring at room temperature (22–23 °C), 10 μl of a stock solution (0.5 mg per ml ethanol) of the fluorescent cyanine dye, 3,3′-dipropylthiodicarbocyanine iodide (designated diS-C$_3$-(5)), supplied by Dr Alan Waggoner of Amherst College, was added. After fluorescence decay had stabilised (approximately 2 min), 10 μl of stock solution of valinomycin was added to promote potassium transport[8]. Finally, increasing amounts of 4 M KCl were introduced into the suspension (Fig. 1). The methods used to record fluorescence have been described before[6]. The data at each circadian time were plotted as the change from the steady state fluorescence (ΔF) in the presence of valinomycin as a function of the

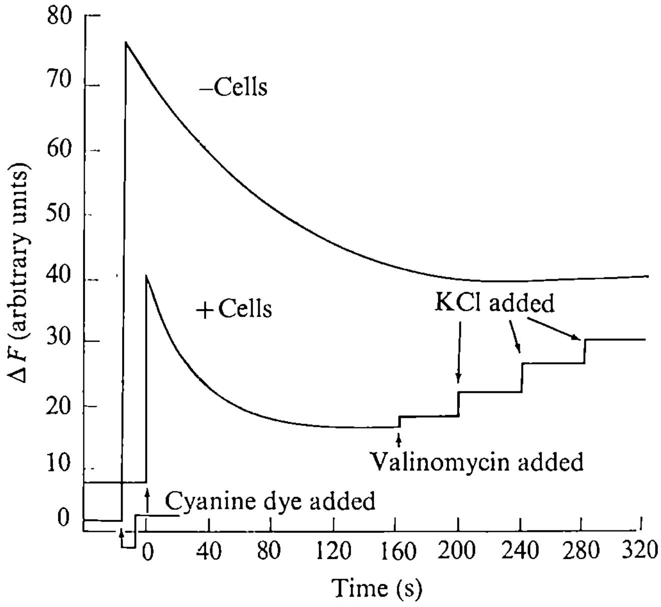

Fig. 1 Schematic of a typical trace of changes in fluorescence intensity of the dye diS-C$_3$-(5) as a function of time in 100 mM NaCl–Tris medium, *p*H 8.1, in the presence (+) and absence (–) of 2.5×10^4 cells ml^{-1}. Excitation was at 622 nm and emission was recorded at 670 nm. Final concentrations of chemicals added as indicated were: dye, $2\,4\times10^{-6}$ M; valinomycin, 6×10^{-5} M, ethanol, 1% v/v, and 64 mM KCl (added as 4-M KCl in 5-, 10-, 50-, and 100-µl increments).

logarithm of the external concentration of potassium $[K^+]_0$.

Gonyaulax, a marine phytoplankter, is normally grown in a medium of approximately 500 mM salt. But at concentrations greater than 300 mM salt, dye fluorescence substantially diminishes. Therefore, a 100 mM NaCl (containing no potassium) solution was chosen for the determinations. In this solution cells were observed not to lyse, or lose potassium (as determined by flame photometry), and maintained their motility.

Gonyaulax has a complex cell covering consisting of several membranes external to the spheroplast[9,10] and it was necessary to determine which membrane the fluorescent cyanine dye probes. When *G. polyedra* cells are centrifuged and stirred, membranes exterior to the spheroplast[10] lose their permeability characteristics. This is readily observable using the mortal stain Evans blue dye[11] and the vital stain neutral red[12]. It seems that the only likely membrane over which a membrane potential could be maintained is the spheroplast-limiting membrane. We observed no change in the steady-state level of dye fluorescent intensity on addition of potassium in the presence of valinomycin to *G. polyedra* cells mechanically ruptured, to isolated cell-wall fractions[10], and to cells maintained for 25 min at 0–10 °C and 30 °C Since the cyanine dyes monitor changes in membrane potential of cells or subcellular organelles only if their limiting membrane is intact[6], we conclude that the dye monitors changes associated with the spheroplast membrane of *Gonyaulax*. Furthermore, in *Amphiuma* red blood cells and Erlich ascites tumour cells, which have internal membranes and organelles, the dye probes the plasma membrane and not the internal membranes[6,13].

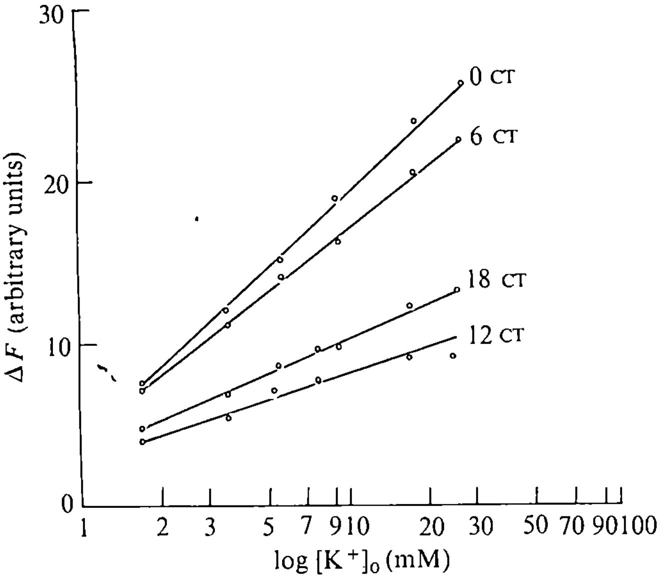

Fig. 2 Change in the steady-state level of fluorescence intensity (ΔF) against $\log[K^+]_0$, conditions as in Fig. 1, at different circadian times. Cells were maintained for approximately 3 d under constant illumination and temperature before and during sampling.

The interaction of the cyanine dyes with membranes and the mechanism by which these dyes monitor the membrane potential are not completely understood[14,15]. But a decrease in fluorescence intensity and a redshift (3–4 nm) in the emission spectrum of the dye is characteristic of the interaction of the dye and biological and model membranes[6,14]. The emission spectrum of the dye diS-C_3-(5) in the presence of centrifuged and stirred *G polyedra* results in a characteristic decrease in the intensity of fluorescence of the dye, and a 4-nm redshift in the emission spectrum.

In previous studies with various preparations, the fluorescence of the cyanine dye decreased in intensity as the potential became more negative (internally), and increased as it became more positive[8]. The decrease was apparently due to binding, and the increase to release of the dye from the membrane[14]. We were not able to observe a decrease in fluorescence intensity indicative of hyperpolarisation on addition of valinomycin to cells in potassium-free medium, although flame photometry showed that addition of valinomycin to cells in K^+-free medium resulted in increased membrane permeability to potassium. The most likely explanation for the lack of a decrease in the intensity of fluorescence with valinomycin in K^+-free medium is that the internal potential was already very negative and that the further hyperpolarisation was not large enough to be detected by the dye. This situation is observed with mitochondria in the energised state[8]. No independent method is available to resolve this possibility. Since a decrease in fluorescence intensity could not be observed, the membrane potential could not be measured by the "null point" method[6], thus whether or not the absolute value of the membrane potential changes with circadian time could not be determined. But the addition of potassium to cells in the presence of valinomycin led to an increase in fluorescence intensity indicative of depolarisation[6]. Plots of ΔF against log $[K^+]_0$ in the presence of valinomycin were linear over most of the range studied (Fig. 2), and the shape of these curves is typical of curves seen with other preparations[8] The relationship suggests that in the presence of valinomycin the potential is shifted towards the K^+ equilibrium potential[8]. Therefore, plots of ΔF against log $[K^+]_0$ in the presence of valinomycin may be indicative of the relative influence of potassium plus valinomycin on the existing potential difference across the membrane[6,8].

Although it is conceivable that oscillations in the concentration and fluorescence intensity of certain pigments could interfere with dye fluorescence emission, no oscillations in the total concentration and fluorescence intensity of chlorophyll *a* and *c* have been observed with circadian time (unpublished results of M.A., B. Prézelin and B.M.S.).

The initial events of cyanine dye–cell–valinomycin interactions (dye fluorescence equilibration time and slight increase in fluorescence with valinomycin (Fig. 1)) appeared constant over circadian time and suggested that the initial attachment of dye and valinomycin was not changing significantly. Plots of ΔF against $\log[K^+]_0$ determined at various times in the circadian cycle generated curves with slopes (linear portion) which varied between two extremes (Fig. 2). A plot of the slope against circadian time showed that the

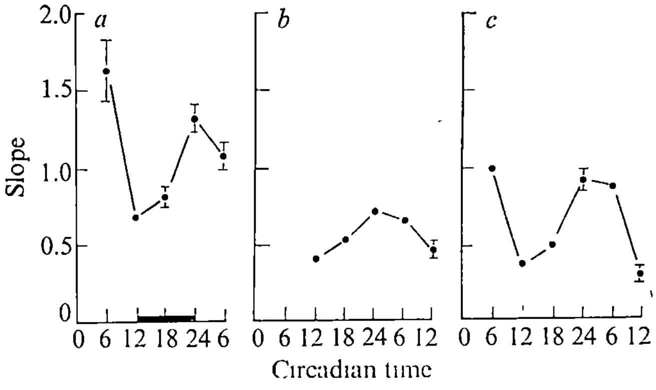

Fig. 3 Plot of the slope, calculated from changes in fluorescence intensity against $\log[K^+]_0$ at various times in the circadian cycle (Fig. 2), as a function of circadian time from three independent experiments. *a*, Cells were maintained on a 12-h light–dark schedule; black bar represents dark period. *b* and *c*, Cells maintained at constant illumination and temperature, 1 and 3 d respectively, before and during sampling. The bar associated with some points represents the extreme values measured Where no bar is shown, the values fall within the circle.

slope varied rhythmically with a 24-h periodicity (Fig. 3). A steep rise in the slope occurred before 0 CT and 24 CT (both corresponding to dawn) followed by a rapid decline observed at 12 CT (corresponding to dusk). The rhythmic oscillation in slope was observed both in a light–dark cycle and after 4–5 d in constant conditions (Fig. 3), characteristic of an endogenous circadian rhythm.

The cyanine dye, diS-C$_3$-(5), seems to monitor some *in vivo* rhythmic phenomenon(a) of the spheroplast membrane, but it is not clear how the changes in slope are to be interpreted. Changes in fluorescence on addition of extracellular potassium and hence changes in slope with circadian time, were observed only in the presence of cells, cyanine dye and valinomycin together, and at approximately room temperature (22–23 °C). When such determinations were made with cells maintained below 10 °C or above 30 °C for 25 min, temperatures at which *Gonyaulax* cells apparently become leaky (unpublished observations of M A. and B.M.S.), plots of ΔF against $\log[K^+]_0$ generated slopes with values near zero. Although this might suggest that, at physiological temperatures (16–24 °C), changes in slope may indicate a circadian rhythm in membrane permeability to ions other than K^+ in the presence of valinomycin, no rhythmic changes in $[Na^+]$, $[Cl^-]$, $[Mg^{2+}]$, and $[Ca^{2+}]$ were detected in *Gonyaulax* by flame photometry (ref. 16 and unpublished data on *Gonyaulax* ion assays of L Anderson and B.M.S.). Furthermore, no change was detected in the steady state of dye fluorescence on addition of increasing amounts of 4 M NaCl to a suspension of cells in the presence or absence of valinomycin. In addition, rhythmic oscillations in total protein, determined by the Lowry[17] and fluorescamine methods[18], were not observed Together these observations suggest that neither the permeability of the membrane to these ions, nor possible rhythmic oscillations in the concentration of non-diffusable anions, can account for the observed rhythmic oscillations in slope.

The constant field equation predicts that the slope of plots of ΔV_m (membrane voltage, ΔF in this case) versus $\log[K^+]_0$ will increase with increasing permeability of potassium relative to other ions[6] In this study valinomycin was used to increase the permeability of potassium specifically. Therefore, it seems that the dye monitors rhythmic changes in the relative influence of potassium plus valinomycin on the existing spheroplast membrane potential, and that these changes reflect rhythmic oscillations in the permeability of the membrane to potassium mediated by valinomycin. The kinetics of valinomycin-mediated K^+ transport are significantly influenced by the dynamic state and chemical composition of the membrane[19–23] and the use of valinomycin-mediated potassium transport has been suggested as a potential probe for membrane structure[24] It is conceivable that the circadian rhythm in valinomycin-mediated transport of potassium and its subsequent effect on the degree to which the membrane can be depolarised implicates a rhythmic alteration in the physicochemical nature of the spheroplast membrane. Whether this includes a rhythmic change in the absolute value of the membrane potential before the addition of potassium and valinomycin could not be determined in this study The existing membrane potential, however, may be differentially influenced by the presence of potassium and valinomycin at particular times in the circadian cycle The existence of rhythmic alterations of the spheroplast membrane is further supported by evidence for a circadian rhythm in the fragility of the spheroplast membrane (unpublished results of M A., B. Prézelin and B.M.S.) and in the composition of the polyunsaturated fatty acids of *Gonyaulax*[25]. Both these circadian rhythms occur at approximately the same phase (0 CT) in the circadian cycle as the rhythmic oscillation in slope observed in this study. Whether these rhythms constitute part of the underlying oscillator or are only driven by it remains to be investigated.

In conclusion, the evidence presented here which suggests an *in vivo* circadian rhythm in the relative influence of potassium and valinomycin on the existing electrical properties of the spheroplast membrane implies a rhythmic temporal reorganisation of the membrane, and supports the suggestion that membrane oscillations are involved in biological clock phenomena. Apart from its significance in the mechanism of the circadian oscillator, a circadian rhythm in membrane temporal reorganisation could account for the large discrepancies in permeability data between laboratories[26], and should be considered in future studies of biological membranes.

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Genetics of chloroquine resistance in malaria parasites

THE resistance of the malaria parasite of man, *Plasmodium falciparum*, to treatment with chloroquine is a growing problem, especially in South-east Asia and South America[1]. It is not known whether the emergence of resistance is attributable to the selection of resistant mutants under drug pressure, to the spread of naturally resistant forms in the parasite population or to adaptation to the drug by previously sensitive parasites. In malaria parasites of rodents, it has been shown that resistance to the antifolate drug pyrimethamine arises by mutation[2] and that the genetic factors involved can undergo recombination with other markers in crosses between resistant and sensitive parasite lines[3]. Resistance to chloroquine in rodent plasmodia seems to take several forms. *P. yoelii* isolates are innately resistant to the drug[4], whereas *P. berghei* and *P. vinckei* are sensitive. Stable chloroquine resistance has been produced in *P. vinckei* by drug selection in the laboratory[5] but resistance developed in this way in *P. berghei* is usually unstable in the absence of the drug[6].

I have investigated the genetic basis of chloroquine resistance in another rodent malaria species, *P. chabaudi*, which I believe serves as a useful model for *P falciparum.* A chloroquine-resistant parasite line (411 AS) of *P. chabaudi* was obtained in mice by submitting blood forms of a sensitive line to a continuous low level drug pressure. Five mice (C57BL, 4–5 weeks old) were each injected with 10^6 blood forms and treated with oral doses of chloroquine at 2 mg kg^{-1} daily for five days from the day of inoculation. Mice were weighed individually at the start of treatment and on day four, the drug dose being adjusted if any change in weight had occurred. Parasites from that mouse which exhibited the highest parasitaemia were injected into a second group of mice and the treatment repeated. Five similar passages were made in mice, the dose being increased to 3 mg kg^{-1} from the third passage. Parasites which survived this course of treatment were then transmitted through mosquitoes into mice and tested for resistance. The line survived treatment of 3 mg kg^{-1} given for six days, whereas the drug-sensitive parent line, which had undergone a similar series of passages without drug pressure, was eradicated

Table 1 Characterisation of 70 clones, produced by mixed infection of mosquitoes by lines 411AS and 96AJ

LDH form*	6PGD form†	Pyrimethamine response‡	Chloroquine response	Number of clones
2	3	S	S	32
2	3	S	R	11
2	3	R	S	0
2	3	R	R	0
2	2	S	S	2
2	2	S	R	7
2	2	R	S	0
2	2	R	R	0
3	3	S	S	1
3	3	S	R	2
3	3	R	S	2
3	3	R	R	1
3	2	S	S	1
3	2	S	R	7
3	2	R	S	0
3	2	R	R	4
Total				70

*LDH2 or 3, Electrophoretic forms of the enzyme lactate dehydrogenase.
†6PGD2 or 3, Electrophoretic forms of the enzyme 6-phosphogluconate dehydrogenase.
‡R, resistant to drug pressure, S, sensitive to drug pressure

The stability of the resistance was tested by keeping the parasite line undrugged for 25 blood passages (6 months), tests for resistance being carried out at passages 10, 15 and 25. At passage 15, parasites were also transmitted through mosquitoes into mice and tested for resistance. In all tests (3 mg kg^{-1} for 6 d) the parasites were found to be resistant, the resistance thus proving stable in the absence of drug pressure.

The genetic basis of the chloroquine resistance was investigated in a cross between line 411AS and a sensitive line denoted 96AJ. The two lines differed additionally in three characters. Line 411AS was resistant to pyrimethamine (15 mg kg^{-1} administered for four consecutive days after inoculation of parasites), and possessed electrophoretic forms of the enzymes 6-phosphogluconate dehydrogenase (6PGD-2) and lactate dehydrogenase (LDH-3) respectively[7]. Line 96AJ was sensitive to both drugs and possessed enzyme forms 6PGD-3 and LDH-2.

The cross was performed using a technique described previously[3]. Equal volumes of parasitised red blood cells of each line were mixed and injected intravenously into a splenectomised rat, in order to obtain increased numbers of gametocytes. Five days later, mosquitoes (*Anopheles stephensi*) were permitted to feed on the mixed infection. At this stage micro- and macrogametes from each line were formed and, as a result, both cross and self fertilisation occurred. Fourteen days later, the infected mosquitoes were permitted to feed on mice. After mosquito transmission, the blood forms which developed were cloned by dilution, and each clone examined for drug response and enzyme type. As controls, the parent lines 411AS and 96AJ were transmitted through mosquitoes separately, and tested for drug response and enzyme type in a similar way. In tests for chloroquine resistance, 10^6 parasites of each clone were injected into two mice, which were treated with six daily doses of chloroquine at 3 mg kg^{-1}. Pyrimethamine resistance was tested similarly using doses of 15 mg kg^{-1} for 4 d. Electrophoretic forms of 6PGD and LDH were detected as in the method of Carter[7]. Seventy clones were derived from the products of the cross, the characteristics of which are shown in Table 1. Clones showing recombinant as well as parental type characters were present. The numbers of clones of the two parental types were significantly different, there being 32 of type 96AJ but only 4 of type 411AS. This suggests that selection against line 411AS had occurred, either when the parental lines were growing together in the rat before mosquito transmission or between zygote formation and the time at which clones were established. No significance, therefore, can be attached to the number of clones found in each recombinant class. It is of interest, however, that the proportion of chloroquine resistant to chloroquine sensitive clones found is 32:38, suggesting that the resistant form has no disadvantage compared to the sensitive form.

The results show that the chloroquine resistance which developed in *P. chabaudi* is a stable character, is inherited in simple Mendelian fashion, undergoes genetic recombination with other markers, and probably arose by mutation and selection in presence of the drug. Although the level of resistance developed is low, the treatment of 3 mg kg^{-1} for six days is sufficient to eliminate sensitive forms, and is similar to the recommended doses for suppression of human malaria[8].

The results, therefore, may provide an explanation of how chloroquine resistant forms could arise in the human malaria parasite, *Plasmodium falciparum*, in areas where the drug is widely used.

Further work is in progress in this laboratory to determine how the genetic factors involved can spread in populations of sensitive parasites. This knowledge is of particular importance as chloroquine resistance in *P. falciparum* is widespread in regions such as South-east Asia, but not yet in Africa.

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Doubling potential of fibroblasts from different species after ionising radiation

IT is well known that chicken fibroblasts invariably die after a certain number of doublings *in vitro* (cell senescence) and that they have never been established by any chemical or physical agent[1]. On the other hand, mouse fibroblasts invariably acquire spontaneously an infinite growth potential, and transformation can be induced at a high frequency by different oncogenes[2]. Other species like man also yield fibroblasts which never acquire spontaneously the capacity to divide for ever[3] but can become permanent cell lines after infection with SV40 virus[4]. Transformation in human fibroblasts has only been unequivocally demonstrated after treatment with certain viruses. This behaviour of fibroblasts *in vitro* has been attributed to different nutritional requirements[5] The two approaches to explain the phenomenon can be summarised as intrinsic properties against environmental conditions. We have previously shortened the lifespans of cultivated chicken fibroblasts with low dose rate ionising radiation[6] We have carried out the same type of experiment with human and mouse fibroblasts and found that the response to ionising radiation fits the relative tendencies of these different fibroblasts to yield permanent cell lines.

Human embryonic lung fibroblast and mouse newborn lung fibroblast cultures were started as described previously[3]. The same nutrient medium (Eagle's MEM with 10% foetal calf serum) was used to cultivate these two types of cell. The initiation and maintenance of chicken embryonic cultures have also been reported previously[6]. Tryptose phosphate broth (5%) was added to the Eagle's medium used to cultivate the chicken fibroblasts, to improve the growth of these cells. The irradiation was started during the phase of active proliferation. The growth curves (Fig. 1) of irradiated chicken fibroblasts were parallel to the growth curve of the controls, but the former had a lower saturation density and stopped dividing earlier. This decrease in the saturation density and lifespan was directly proportional to the number of radiation dose received.

In human fibroblasts the saturation densities of the irradiated cultures oscillated around the saturation density of the controls. Cultures irradiated with 100 rad, however, stopped dividing earlier than the controls. The cultures irradiated with 200, 300 and 500 rad had the same lifespan as control cultures, and the cultures irradiated with 400 rad had the longest survival.

With mouse cells the establishment of the control cultures followed the pattern described previously[7]. First, there was a progressive decline in the maximal number of cells (time when the number of mitoses was negligible); around passage 9 the cells recovered from the growth decline, the maximal cell density increased progressively and finally the population became established with a saturation density that stabilised around 10^5 cells cm^{-2} from passage 20 onward The growth curves of the irradiated cells were of the same type as in the controls but the recovery occurred earlier.

Calculation of the total amount of cells produced in controls and in irradiated cultures gave different results in each species (Fig. 2). With chicken fibroblasts the total amount of cells produced in each group decreased logarithmically with increasing doses of radiation received. With human fibroblasts the total number of cells was identical in all groups so that there was no slope. Finally, with mouse fibroblasts the total amount of cells produced increased logarithmically and was directly related with the radiation dose received.

These results indicate that ionising radiation accelerates a natural phenomenon: in cells with a limited growth potential (chicken) it shortens the lifespan and in cells that can acquire an unlimited growth potential (mouse) it

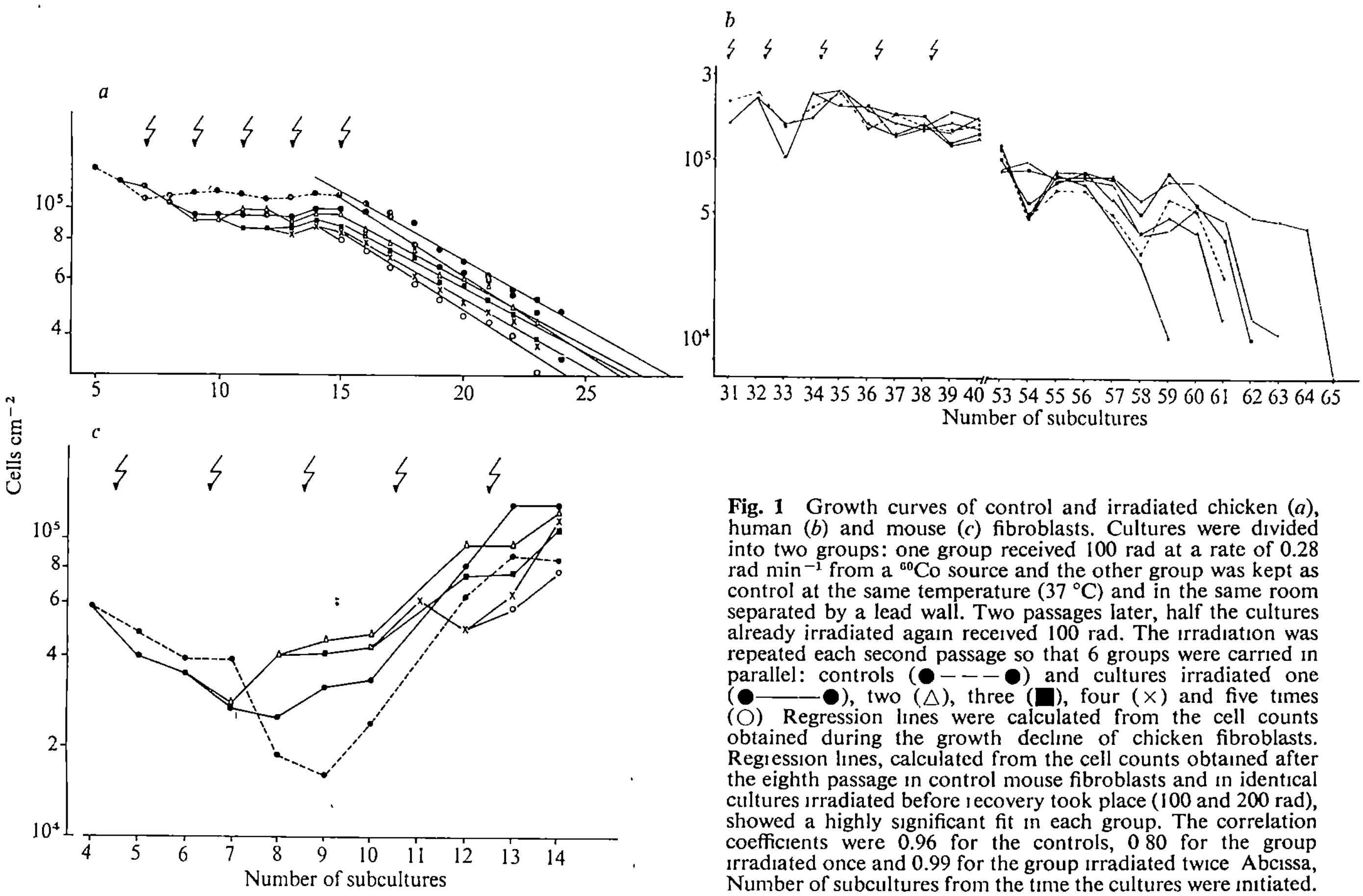

Fig. 1 Growth curves of control and irradiated chicken (*a*), human (*b*) and mouse (*c*) fibroblasts. Cultures were divided into two groups: one group received 100 rad at a rate of 0.28 rad min^{-1} from a ^{60}Co source and the other group was kept as control at the same temperature (37 °C) and in the same room separated by a lead wall. Two passages later, half the cultures already irradiated again received 100 rad. The irradiation was repeated each second passage so that 6 groups were carried in parallel: controls (● – – – ●) and cultures irradiated one (●——●), two (△), three (■), four (×) and five times (○) Regression lines were calculated from the cell counts obtained during the growth decline of chicken fibroblasts. Regression lines, calculated from the cell counts obtained after the eighth passage in control mouse fibroblasts and in identical cultures irradiated before recovery took place (100 and 200 rad), showed a highly significant fit in each group. The correlation coefficients were 0.96 for the controls, 0.80 for the group irradiated once and 0.99 for the group irradiated twice Abcissa, Number of subcultures from the time the cultures were initiated. Each point on the curves represents the means of 3 cell counts carried out with a Coulter Counter after trypsinising the cultures before subcultivation. These curves represent the number of live cells present in the cultures before each subcultivation. Since at each passage the inoculum is always half of the number of cells found at resting stage (1:2 split), the curves merely express if the population doubled or not after each subcultivation. Number of non-dividers not known.

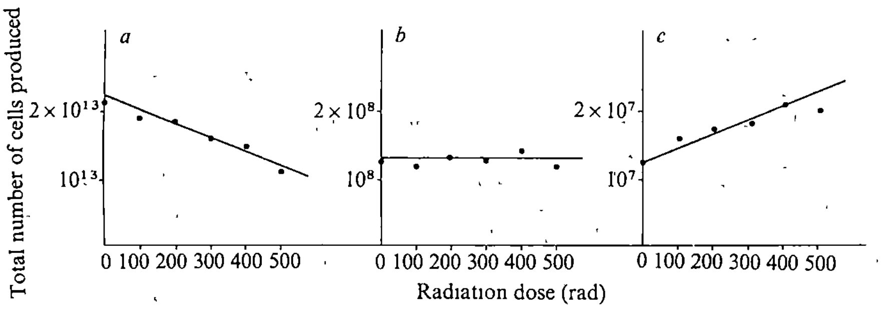

Fig. 2 Total amount of cells produced in control and irradiated cultures in the experiments illustrated in Fig. 1. In mouse fibroblasts the total number of cells produced was calculated up to passage 20—that is, at the time when the saturation densities became stabilised and identical in all groups. *a*, Chicken; *b*, human; *c*, mouse.

accelerates the acquisition of the latter; human fibroblasts showed an intermediate response since ionising radiation neither established the cultures as with mouse cells nor reduced the number of cells produced, as with chicken fibroblasts. Human cells also show an intermediate behaviour to chicken and mouse fibroblasts in the sense that chicken cells never establish either spontaneously or after treatment with virus, chemicals or radiations, mouse cells establish spontaneously, and human cells never establish spontaneously but can establish after infection with certain viruses. So they seem more prone to become established than chicken cells, but less than mouse cells.

It is difficult to see what could cause the different responses of the fibroblasts from these three species. It seems, however, that there are three possible explanations. It is possible that in the case of mouse fibroblasts there are some cells in the original population which have the potential of dividing indefinitely. If these cells are less radiosensitive, ionising radiation could favour their overgrowth by killing or retarding the growth of the other cells in the population which have a limited doubling potential. In that case, one would have to infer that in chicken fibroblast populations there are not cells with an infinite doubling potential. On the other hand, among human fibroblasts there would be clones with different growth potentials, and ionising radiation would favour the clones with the longest division potential.

The other hypotheses would concern the presence or absence of repair mechanisms or of a reparable substrate. It has been suggested that malignancy could be due in certain cases to mutations caused by faulty repair and that situations inducing repair therefore increase malignant transformation[8]. Repair induced by low dose rate ionising radiation would cause mutations which determine the capacity to divide indefinitely. The situation would be analogous to what happens with *Micrococcus radiodurans* that becomes resistant to ionising radiation, because it develops the capacity to repair quickly the DNA lesions[9]. If this were the case one would have to postulate that cells like mouse fibroblasts are endowed with the capacity to spontaneously undergo these changes, which would be accelerated by ionising radiation. Cells like chicken fibroblasts, on the other hand, are endowed with a finite lifespan, perhaps because they have no repair enzymes or because the substrate (for example, DNA) has a molecular organisation which would impede the action of these enzymes. In any case the results seem to provide further evidence that a finite or infinite lifespan is an intrinsic property of cells and is not just dependent on a particular environment.

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Absence of oxygen effect for induction of resistance to ionising radiation

Survival curves for *Chlamydomonas reinhardii* exposed to ionising radiation have an initial 'shoulder' region where sublethal damage is accumulated, followed by an exponential region where a further single event will result in cell death. When an initial dose is given to cells, sufficient to reduce the surviving fraction to the exponential region, followed by a series of graded doses given some hours later, cells recover from sublethal injury inflicted by the first dose, as shown by the return of the shoulder region on the second dose survival curve. The slope of the exponential region of the second dose survival curve is smaller than that of a single dose survival curve[1]. Thus cells have become more resistant as a result of exposure to a

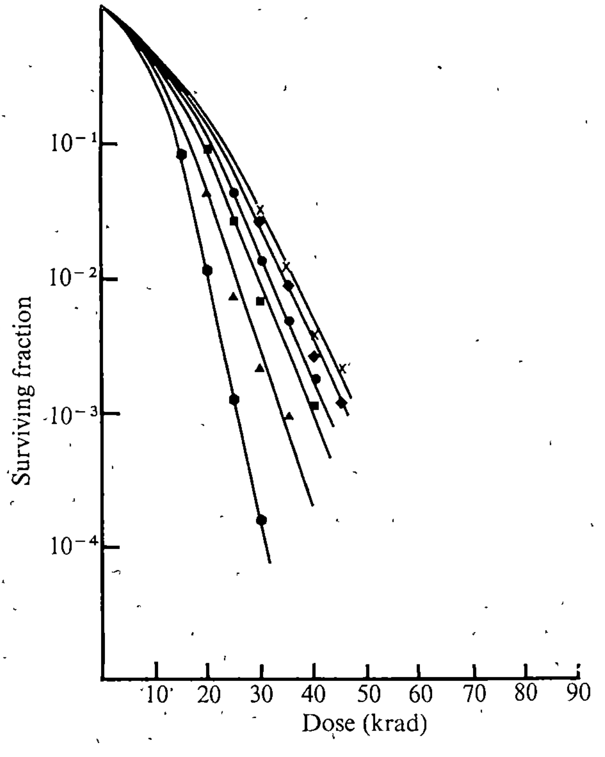

Fig. 1 Survival curves for *Chlamydomonas* given either single doses (●) or fractionated doses at different times after a first dose of 15 krad given in anoxic conditions 1 h (▲), 2 h (■), 3 h (●), 4 h and 5 h (×) between doses Split dose curves have been normalised with respect to the surviving fraction after the first dose.

first dose. A similar effect has been reported for a line of HeLa S3 cells[2]. The increase in resistance observed in these two types of cell has been interpreted[1] as resulting from an increase in the capacity of cells to repair radiation damage. An alternative hypothesis[3] that could account for the observed change in sensitivity proposes that cells sublethally damaged by the first dose of radiation are repaired during the interval between doses, so as to render the intracellular sites less susceptible to damage from a second dose of radiation. The experiments reported here cast doubt on the validity of the latter hypothesis.

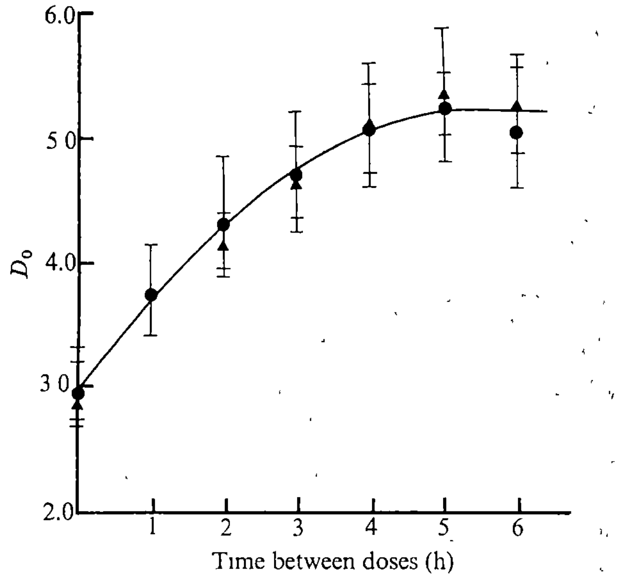

Fig. 2 D_0 as a function of time between doses given in aerobic conditions after a first dose of 15 krad given in aerobic (▲) or anoxic (●) conditions. Vertical bars represent 95% confidence intervals

I also report that irradiation of cells in aerobic or anoxic conditions was equally effective in inducing an increase in resistance of cells to a second series of doses. That is, the oxygen enhancement ratio (OER) was 1.0 for induction of resistance, in contrast to the OER of 2.2 observed for cell killing. The OER for cells killed by a second series of doses given some hours after an initial dose was the same as that for cells killed by single doses.

Alper has postulated that radiation causes two types of cellular lesions both of which can contribute to cell death[4,5]. One, called type N, occurs in the DNA of the cell and is subject to little or no enhancement by oxygen while the other, called type O, occurs in cell membranes and is subject to a large enhancement by oxygen. It has been postulated that after irradiation differential repair can occur in these two intracellular regions, resulting in variations in resistance of cells and in the OER for cell death. Radioresistance may thus result from repair to DNA which will result in a large OER value because killing will be due to events occurring in the cell membranes or membrane-associated structures. Radioresistance may also result from repair to membranes or membrane-associated structures which will result in a lower OER because killing will be due predominantly to events occurring in the DNA. Alper has shown for variants of *Escherichia coli* strain B that a proportionality exists between the resistance of the variants to radiation and their OER values, and has postulated that this occurs because the more resistant bacteria can repair type N damage. Wild-type forms of *C. reinhardii*[6] and the slime mould *Dictyostelium discoideum*[7] have values of OER of about 2.2 and 1.8 respectively whereas their radiosensitive mutants have values of OER of 1.0 or less. The more radioresistant wild types of these organisms were also shown to be resistant to ultraviolet light of a wavelength absorbed strongly by nucleic acid, suggesting that the resistance in these cases to ionising radiation may result from the cells' ability to repair damage to DNA.

In the experiments reported here the resistance of *Chlamydomonas* to a second series of doses was increased by giving an initial dose of radiation followed by an interval before the second dose. It was therefore surprising, in view of the results mentioned above, to find that the OER was unchanged for these more resistant cells.

The methods used in the culture of vegetative cells of *C. reinhardii* have been described previously[8,9]. Cells were irradiated in suspension in a liquid medium held in a glass vessel and bubbled with either air or nitrogen (BOC white spot grade). To achieve anoxic conditions, nitrogen was bubbled for 10 min before and during irradiation. Irradiations were performed in the dark. Samples were irradiated with 7 MeV electrons from the MRC linear accelerator at a dose of 20 krad min^{-1}. Doses were monitored with an ionisation chamber which had been calibrated against a Baldwin-Farmer secondary standard dosemeter, and checked by the ferrous sulphate method.

Table 1 Oxygen enhancement ratios for cells given single and split doses

Treatment	Oxygen enhancement ratio
Single doses	2.18 (2.00–2.37)
Second series of doses following an initial conditioning dose of 15 krad given in aerobic conditions	2.25 (2.06–2.46)
Second series of doses following an initial conditioning dose of 15 krad given in anoxic conditions	2.22 (2.08–2.36)

Figures in parentheses are 95% confidence intervals.

During intervals between doses, suspensions of cells were shaken gently in conical flasks in the light at 22 °C. After irradiation and appropriate dilution, cells were plated on membrane filters (Oxoid) resting on yeast extract agar (Oxoid). Colonies were scored after 3 d of growth under continuous illumination.

Experiments in which cells were given a first dose in either aerobic or anoxic conditions, followed by a graded series of doses in aerobic conditions at various times (Fig. 1), showed that the final slopes of the split dose survival curves decreased

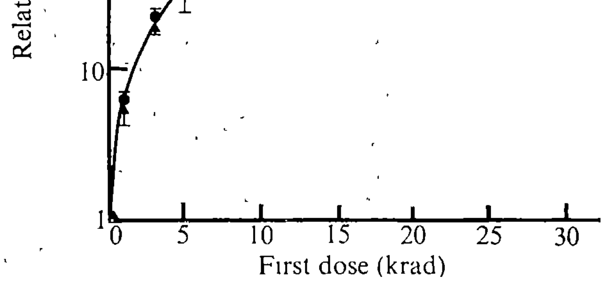

Fig 3 Relative survival as a function of first dose given either in aerobic (●) or anoxic (▲) conditions Cells were given various first doses in either aerobic or anoxic conditions followed by a second 'test' dose of 30 krad given in aerobic conditions 5 h later. Survival after the two doses was expressed as a fraction of cells surviving after the first dose Relative survival values were then calculated by dividing the surviving fraction after the two-dose treatment by the surviving fraction after a single dose of 30 krad given in aerobic conditions

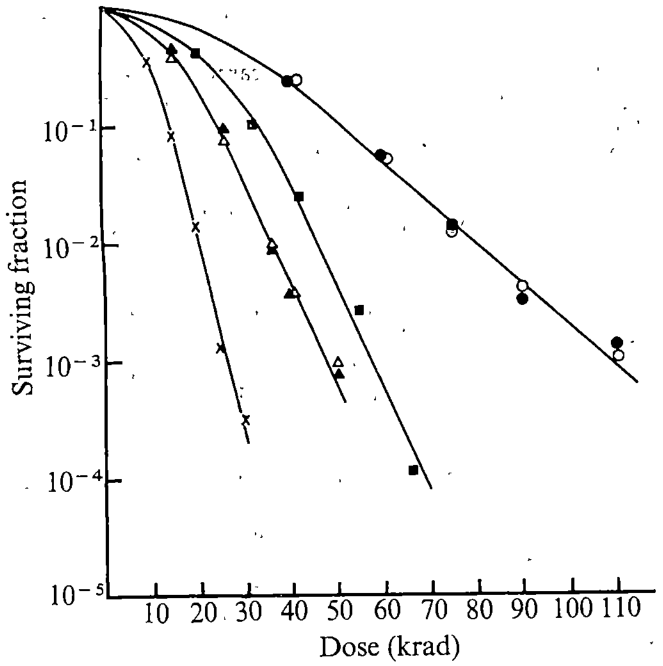

Fig. 4 Survival curves for cells given either single doses in aerobic conditions (×) and anoxic conditions (■), or a series of graded doses given 5 h after an initial dose of 15 krad given in aerobic conditions (open symbols) and in anoxic conditions (closed symbols) Second doses were given either in aerobic (▲ and △) or in anoxic conditions (● and ○). Surviving fractions of cells after two-dose treatments were normalised with respect to the surviving fraction after the first dose of 15 krad.

progressively up to 5 h after the first dose was given, the D_0 value changing from 2.8 krad for the single dose curve to 5.3 krad for the split dose curve at 5 h. The pattern of increase in D_0 with time was the same whether cells had been irradiated in aerobic or anoxic conditions (Fig. 2). The increase in resistance was also dose dependent (Fig. 3) up to about 15 krad, above which resistance reached a plateau. The dose dependence of the increase in resistance was the same whether the first doses were given in aerobic or anoxic conditions. Thus the OER for the induction of an increase in resistance was 1.0, a surprising result since the OER for cell killing was 2.2 (Table 1). This lack of an oxygen effect for the induction of resistance suggests that the initial damage causing the induction may be registered in a type N site that is in DNA. Lower OER values have been obtained for the induction of mutations than for cell killing in other cell types[10-12] although the values of OER for mutation induction were always somewhat larger than 1.0. In other cases quite large values of OER have been obtained for processes involving DNA such as the induction of β-D-galactosidase enzyme[13], and the induction of prophage[14].

The OER for killing *Chlamydomonas* was the same for cells given a second series of doses after an initial dose as for cells given single doses (Table 1). Figure 4 shows the survival curves for 'recovered' cells which had been given an initial conditioning dose and for cells given single doses. After an initial dose of 15 krad in aerobic conditions the surviving fraction of cells was about 8% whereas after a first dose of 15 krad given in anoxic conditions the surviving fraction was about 60%. Thus in cells given an initial dose of 15 krad in anoxic conditions only a small accumulation of sublethal damage had occurred, whereas in aerobic conditions the same dose produced the maximum amount of accumulated sublethal damage. Yet the increase in resistance was the same for the two different conditions prevailing at the time of the first dose. The increased resistance could not therefore be attributable to changes in the physical or chemical nature of the sublethally affected sites during their repair since the extent of the repair of sublethal damage in the two cases was very different. The results suggest that the increase in resistance may be attributable to an increased capacity of cells to repair damage after a first dose has been given. It was shown in an earlier publication[3] that the increased resistance could not result from more rapid repair of sublethal damage during the second irradiation, that is from a very rapid dose rate effect.

The constancy of the OER observed when the increased resistance was induced (Table 1) suggests that the balance of repair of type N and type O damage in cells remains the same. For the OER to remain constant in the more resistant cells the amounts of both enzymes (which are presumably normally present in cells) responsible for repair of the two types of lesion would have to be increased simultaneously and in proportion to the amounts normally present, so enlarging the cellular pool of both repair enzymes. It seems improbable that this could result from independent direct action of radiation on the two relevant gene sites, but could perhaps result from a 'message' passed from the initial site of radiation damage in the DNA to the two genes responsible for type O and type N enzyme synthesis. Earlier experiments[15], which showed that the decrease in sensitivity of cells can be prevented by treatment of cells during the interval between doses with inhibitors of protein synthesis, lend some support to the idea that increased resistance results from the increased synthesis of repair enzymes.

In conclusion, the results presented here show that the increased resistance of cells induced by a conditioning dose of radiation depends on both time and size of the initial 'stimulating' dose given. The lack of an oxygen effect for the induction of resistance suggests that the process occurs as a result of the interaction of radiation with the DNA of the cell.

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Chemical mutagen hypersensitivity in ataxia telangiectasia

THE growth of knowledge about DNA replication and repair in bacteria has been aided by the availability of a series of mutations specifically affecting these processes. Almost invariably, defects in repair processes result in an alteration in the sensitivity of cells to ionising radiation and other mutagenic agents. Knowledge is much less advanced for animal cells, although information has been obtained using cells from patients with, for example, xeroderma pigmentosum which have provided six possible mutants in DNA repair[1,2]. The diseases classified as chromosome breakage syndromes—ataxia telangiectasia (AT), Fanconi's anaemia and Bloom's syndrome—may also provide a rich source of DNA metabolic mutants. The frequent association of defective DNA metabolism with mutagen sensitivity has suggested an approach to the study of chromosome breakage syndromes. Finkelberg *et al.*[3] have found that Fanconi's anaemia cells are abnormally sensitive to mitomycin C (MC). We have now used this approach to determine whether cells from AT patients are potentially defective in DNA metabolism. We examined their sensitivity to actinomycin D, MC and methyl methane sulphonate (MMS), and the results indicate that AT cells are clearly more sensitive to actinomycin D and that responses to MMS and MC are more variable.

The cell lines used were obtained from skin biopsies by standard explant techniques[4] and had been stored in a liquid nitrogen freezer before use. All had undergone less than 20 doublings after primary outgrowth. Table 1 lists the suspected genotype and other pertinent data for these cell lines. All cultures were maintained in an α medium[5]. Except where specifically noted, the α medium was supplemented with 15% foetal calf serum (FCS) (Flow Laboratories). Concentrations of nucleosides were 10 μg ml^{-1} each, of streptomycin, 100 μg ml^{-1} and of penicillin, 100 U ml^{-1}. Cultures were incubated in an atmosphere of 5% CO_2 in air at 37 °C.

Cells were collected and subcultured after washing once with phosphate-buffered saline (PBS) using 0.25% trypsin (Difco) in citrate saline. The action of trypsin was stopped with 5 ml medium containing FCS.

The plating efficiency (PE) of the AT lines was somewhat lower than that of the other lines (Table 1). Although the lower PE and (we assume) associated poor growth led to rather diffuse colonies of quite varied sizes, it did not contribute to the observed mutagen sensitivity. The killing agents we used do not seem to prevent cells from attaching to Petri dishes; thus "dead" cells remain (much as a feeder layer) and contribute to a high background when high cell densities are plated. In our experiments all treatments were performed on log phase cells at an average density of 5×10^5 cells per Petri dish (100 mm diameter). With both a low PE and a maximum of approximately 5×10^5 cells per treatment point, it is impossible to carry the killing curves much beyond 2.5 logs. This difficulty could be overcome by treating larger numbers of cells. We found, however, that with smaller doses of the drugs (that is, at relatively high survival) a greater degree of reproducibility was attained. Abnormal responses were observed readily in these conditions. For example, with larger doses of MMS (30-min exposure) the average relative survival for eight independent control lines was 0.013 (s.e. 0.0057), yet in eight separate experiments with AT lines, we observed no survivors.

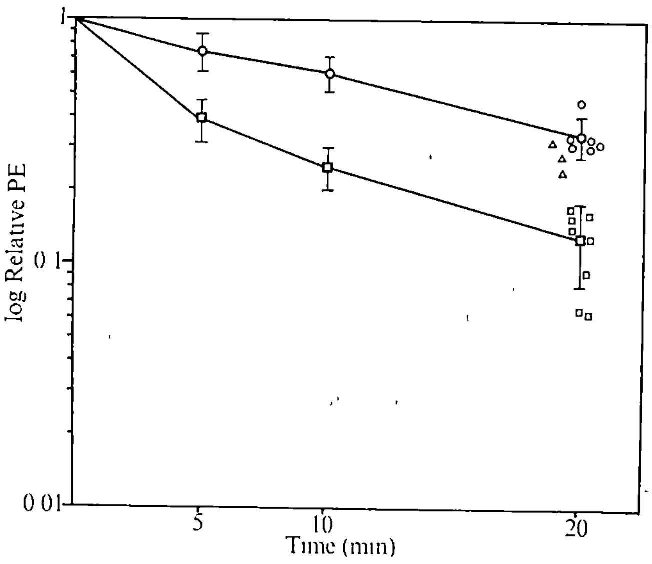

Fig. 1 Killing by actinomycin D of AT (□), HET (△) and control (○) cell lines Cultures to be treated were seeded in replicate at 2.5×10^5 cells per Petri dish (diameter 100 mm) 48 h before treatment At zero time the medium was removed and replaced with medium containing actinomycin D (1 μg ml^{-1}) After prescribed incubation periods at 37 °C, the mutagen-containing medium was aspirated, and the cells were washed once with medium lacking serum and then once with PBS Treated and untreated cells were collected as described in the text and counted in a particle counter (Particle Data Inc) Cells were diluted and five replicates at each dilution were plated in dishes (100 mm diameter) and incubated for 2 weeks before staining with 1.0% methylene blue in 70% isopropanol With the AT cell lines many colonies were very poor, and so all scoring of colonies in control and AT lines was checked under a dissecting microscope Only colonies containing 32 or more cells were counted. The error bars represent 95% confidence limits on 6 separate control cell lines. *Mycoplasma* testing was performed on all cultures before the experiments All the lines used proved to be uncontaminated

Cells from eight AT patients from six sibships and from three related heterozygotes (HET cells) together with six control lines (from normal subjects) were tested for sensitivity to actinomycin D. Figure 1 shows the data for the AT and control lines (with 95% confidence limits). Figure 1 includes data from two repeat experiments with two control lines and the values for the three heterozygotes, one tested twice and averaged. To indicate the variability between lines, all the data points obtained after treatment with actinomycin D are shown in Fig. 1 for 20 min of exposure.

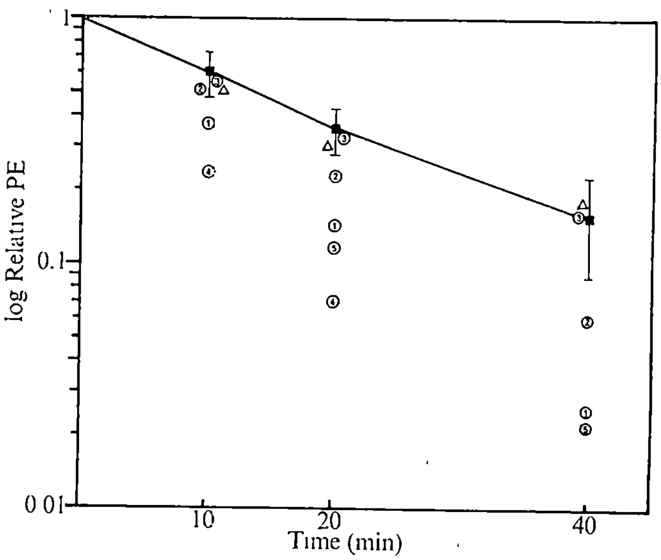

Fig. 2 Killing by MC of five AT lines with six controls (one repeated twice) and one heterozygote. Techniques were as for Fig. 1, with MC at a concentration of 1 μg ml^{-1} (Kyowa, Hakko, Kogyo Co.). Error bars are as in Fig. 1 ■, Controls (7); 1, AT194 (2); 2, AT95 (2); 3, AT97 (2); 4, AT416 (1), 5, AT181 (3); △, HET96 (1). Numbers in parentheses refer to numbers of experiments (values averaged).

Five AT cell lines, six control (one repeated twice) and one heterozygote were tested for MC sensitivity As shown in Fig. 2, four AT lines are significantly more sensitive while the fifth, AT97, was similar to controls. Figure 3 shows MMS killing curves. In Figs 2 and 3 values are not presented for all cell lines at 5 min and in the experiments shown in Fig. 2 we did not observe any survivors for AT416 after 40 min of exposure to MC.

AT95 and AT97, which are derived from sibs, differed in their degree of sensitivity to MMS. Cells from one parent of these sibs were tested and found to be intermediate in sensitivity.

The inherent ability of a cell to survive in culture depends on its ability to correct or tolerate errors generated during normal DNA replication, recombination and/or repair. The results shown in the three figures indicate that cells derived from AT patients have a greater sensitivity than normal to various chemicals known to interact with DNA. This is not the result of their low PE· comparison of the PE and degree of sensitivity of the AT lines (Table 1, Figs 2 and 3) shows a lack of correlation. In addition, experiments we have performed indicate that cell lines with a PE as low as 0.02 can have normal sensitivity to these chemicals.

Actinomycin D is an antibiotic that inhibits both RNA and DNA synthesis by binding directly to DNA[6]. It also has cell-cycle-specific cytotoxicity similar to that of γ radiation[7] and produces γ-ray-like breaks in the DNA of cultured KB cells[8]. Studies with *Drosophila* have shown that besides the inhibitory action on RNA synthesis[9], actinomycin D stimulates recombination specifically in the region of the centromere[10]. That is why we examined actinomycin D sensitivity in AT cells

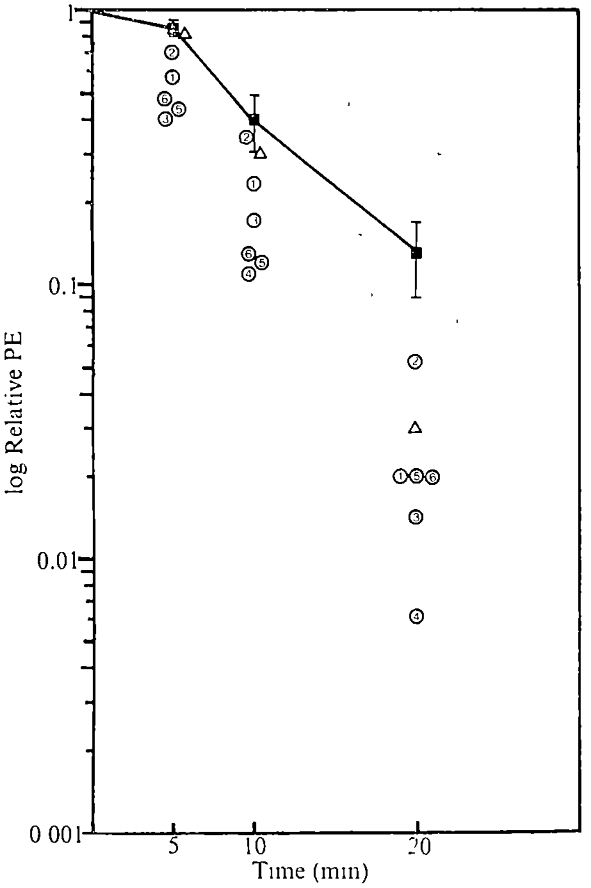

Fig. 3 Killing by MMS of six AT lines, six controls (two repeated twice) and one heterozygote. Techniques were as for Fig 1, with MMS at a concentration of 500 μg ml^{-1} (Eastman Kodak). Error bars are as in Fig. 1 ■, Controls (7); 1, AT194 (1); 2, AT95 (2); 3, AT97 (2); 4, AT416 (1), 5, AT181 (1); 6, AT81 (1); △, HET96 (1) Numbers in parentheses refer to numbers of experiments (values averaged)

Eight AT lines from six different sibships had a significantly impaired capacity to survive low doses of the antibiotic. In addition to generalised sensitivity to actinomycin D, most of the AT lines were also sensitive to MC and MMS. Although not all cell lines have been tested with all three drugs, the apparent normal response of AT97 to MC suggests that other, possibly familial, factors influence this sensitivity. The differences in the sensitivity of AT95 and AT97 (Fig. 3) are consistent with this idea We have observed intrafamilial differences in the degree of MC, actinomycin D and MMS sensitivity (unpublished results) and the combination of the AT genotype with a more, or less, sensitive input from one parent could produce the observed results. This explanation can be confirmed only by a study of the second parent in this sibship. It is possible that the variation in mutagen sensitivity and PE observed with the AT lines used is a reflection of heterogeneity in this disorder. Heterogeneity has been well documented in xeroderma pigmentosum[1,2] and preliminary studies indicate heterogeneity at the biochemical level in AT (M. C. Paterson, personal communication).

Table 1 Plating efficiency of the cell lines

Cell line	Sex	Age (yr)*	PE	Genotype	Relationship†
57	F	21	0 53	Control	—
62	M	35	0.35	Control	—
68	F	40	0 46	Control	—
160	F	5	0 56	Control	—
162	M	13	0 55	Control	—
192	M	2	0 67	Control	—
80	M	40	0 51	HET	Parent of 81
81	M	0.75	0.11	AT	—
82	M	14	0 40	AT	—
95	F	10	0 31	AT	Sib of 97
96	F	47	0.41	HET	Parent of 95, 97
97	M	16	0.27	AT	Sib of 95
180	M	7	0.10	AT	Sib of 181
181	M	2.5	0 19	AT	Sib of 180
193	F	31	0 39	HET	Parent of 194
194	F	9	0.098	AT	—
416	M	4	0.23	AT	—

The control lines were obtained from the Genetics Department, Hospital for Sick Children and the AT and HET lines were provided by Dr M Thompson of the same institution PE, average plating efficiency obtained for log phase cells at zero exposure (see Fig. 1 legend)

*Age at time of biopsy

†Relationship between heterozygotes and patients.

The apparent similarities between the action of AD and γ radiation suggest that AT cells should show enhanced sensitivity to γ rays, this has been confirmed with three different AT cell lines[11].

It is believed that MC-induced cross links are repaired by a combination of excision repair and post-replication repair[12]. The defect in the AT cells which show MC sensitivity could be in either (or both) repair systems or possibly in DNA synthesis.

Observations made with microorganisms indicate that excision repair is a highly accurate system, while post-replication repair is error prone[12]. We have performed preliminary Luria-Delbruck fluctuation analysis on one AT line and found an increased frequency of spontaneous 6-thioguanine-resistant variants This observation is consistent with an excision defect in AT cells. Such a defect has been confirmed and some AT cells seem to be deficient in γ-ray lesion-specific endonuclease[13].

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Frequency of ultraviolet light-induced mutations is higher in xeroderma pigmentosum variant cells than in normal human cells

PATIENTS with the inherited disease, xeroderma pigmentosum (XP), are subject to multiple carcinomas of the skin on areas exposed to sunlight[1]. Fibroblasts from the skin of the majority of these patients (classical XP) have been shown to be deficient in excision repair of lesions induced in DNA by ultraviolet radiation[1-3]. One group of patients, however, has been designated 'XP variants' because, although they suffer the clinical manifestations of the disease, they carry on normal excision repair of such ultraviolet-light damage to DNA[4-6]. Lehmann *et al.*[6] reported that cells from such XP variants are abnormally slow in converting initially low molecular weight DNA, synthesised after ultraviolet irradiation, into high molecular weight DNA similar in size to that produced in unirradiated cells. Although these authors suggest that such abnormal DNA replication might explain why such patients are susceptible to cancer of the skin, they do not propose any mechanism. If the somatic cell mutation hypothesis on the origin of cancer, first suggested by Boveri[7], is correct one would expect the frequency of mutations induced by ultraviolet light to be higher in cells derived from skin biopsies from both classical and variant XP patients than in cells from normal persons. To test this hypothesis, we have carried out a series of quantitative investigations comparing the frequency of ultraviolet-light induced mutations to azaguanine resistance in normal human skin fibroblasts with that found in various strains derived from classical and variant XPs. We have found that cells derived from both kinds of XP patients indeed show much higher frequencies than normal cells. The data comparing two classical XP strains with normal cells have been published[8,9]. Here, we present data obtained with variant strain, XP4BE.

Figure 1 shows that this strain is more sensitive than normal cells to the killing effect of ultraviolet radiation. These particular cell survival data, taken from the cytotoxicity assays which accompanied each mutagenicity experiment, confirm our earlier reports[8-11] that XP variants are killed by lower doses of ultraviolet light than normal

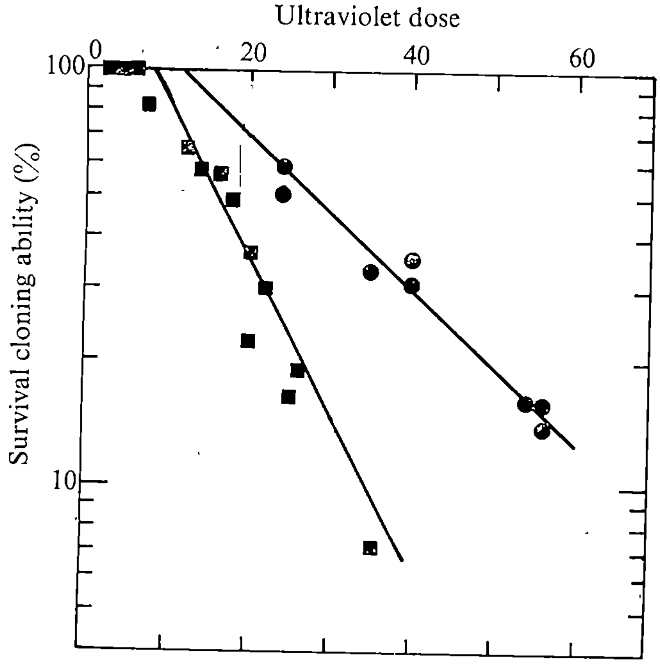


Fig. 1 Comparison of the cytotoxic effect of ultraviolet irradiation as a function of dose, in normal cells (●) with that in the variant, XP4BE (■), as determined from the percentage survival of the colony-forming ability. Survival of the cloning ability of irradiated cells divided by that of unirradiated controls is expressed as a percentage Each symbol represents the percentage survival averaged from 12 replicate dishes of cells receiving the specified dose. Procedures for determining the cytotoxicity of ultraviolet radiation have been described[8,10]. These survival data are taken directly from the cytotoxicity experiments accompanying each of the mutagenicity experiments shown in Fig 2. Lines are calculated by the method of least squares.

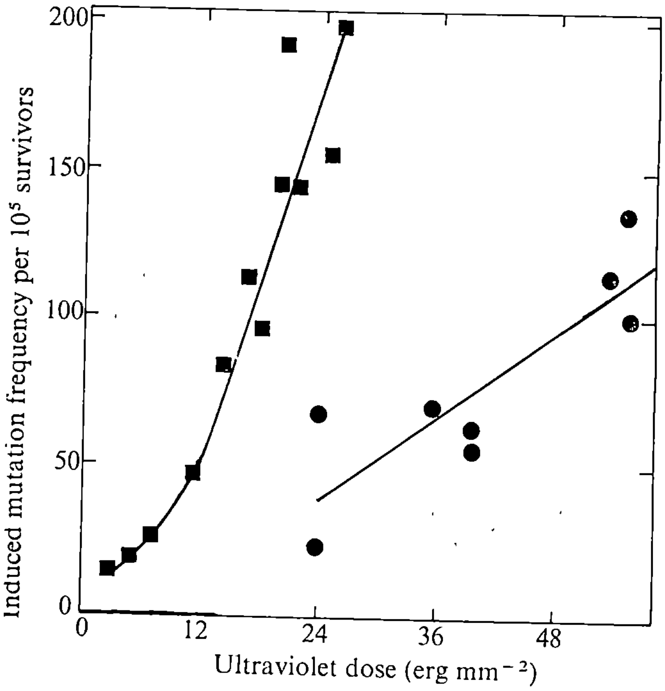
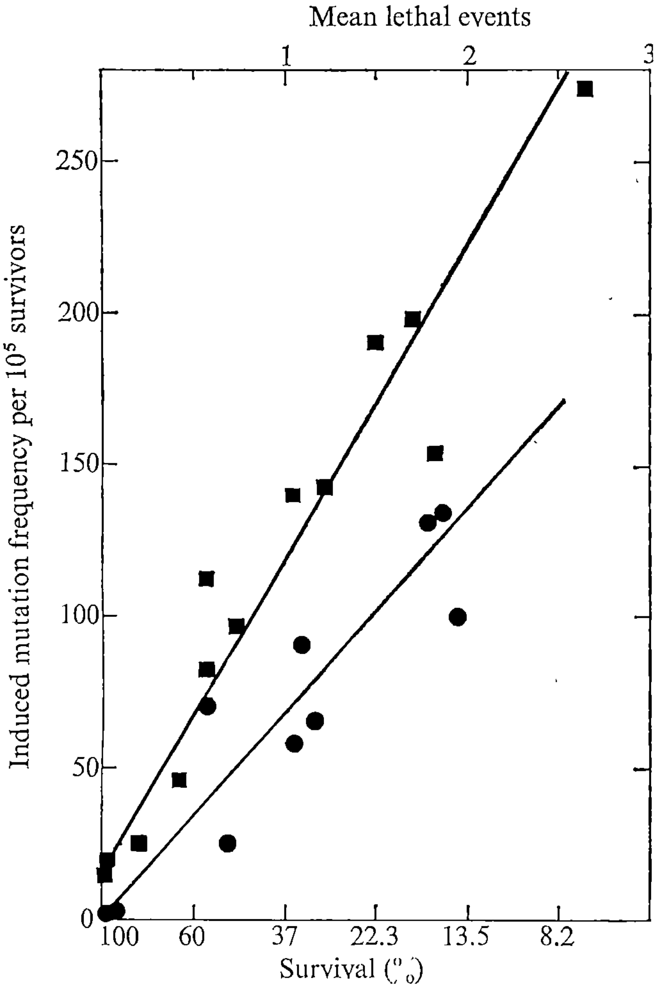


Fig. 2 Frequency of mutations to azaguanine resistance induced in normal human cells (●) and in XP4BE (■) as a function of the dose of ultraviolet radiation administered. The straight line portion of these curves was calculated by the method of least squares. For each experimental point, populations of $1-3\times10^6$ cells were plated in individual dishes at $0.7-18\times10^3$ cells per dish, allowed 10–12 h to attach, rinsed with phosphate-buffered saline, irradiated as described and given Ham's F10 medium supplemented with 15% foetal calf serum. Cells were allowed sufficient expression time to undergo three cell divisions to 'fix' the mutation and permit the necessary diluting out of pre-existing hypoxanthine (guanine) phosphoribosyl transferase enzyme. Cells were then given selection medium 1(F0 with 15% calf serum supplemented with 2×10^{-5} M azaguanine) three times weekly for about 28 d until macroscopic azaguanine resistant colonies appeared. Each mutation experiment was accompanied by a cytotoxicity assay to determine cell survival and a reconstruction experiment with azaguanine resistant Lesch Nyhan cells[12-14] to determine the efficiency of recovery of mutants (see, for example, refs 12 and 13). The induced mutation frequencies observed in each strain have been corrected for the cloning efficiency of the particular strain to make a valid comparison between strains with different cloning efficiencies (see, for example, ref. 14). In the above experiments the cloning efficiencies ranged from 7–15% for XP4BE and from 15–25% for normal cells The frequencies of spontaneous or pre-existing mutants in each of the experimental populations used were comparable. (This is also a requirement if a valid comparison is to be drawn between any two strains.) Fifteen independently isolated azaguanine resistant colonies of either cell strain were tested. All of them were able to form colonies in azaguanine after being cultured in the absence of this selective agent for ten or more cell doublings; 73% exhibited levels of hypoxanthine–guanine phosphoribosyltransferase activity less than 15% of normal cells (see, for example, ref. 12 for enzyme assay). A dose of 2×10^{-5} M azaguanine in our experimental conditions (serum concentration, and so on) was demonstrated to prevent an exponentially growing non-resistant population from attaining more than a 0.20 increase in cell number and to lower the survival of the colony-forming ability of the population to 0 01–0 001% of the unirradiated control.

cells even though both kinds of cells are reported to have equal rates of DNA excision repair. The corresponding mutagenicity results for these strains are shown in Fig. 2. The XP4BE strain exhibits a significantly higher than normal frequency in induced mutations per dose of ultraviolet radiation These results provide strong support for the somatic cell mutation hypothesis on the origin of cancer.

The mechanism by which such increased mutagenicity occurs in the variants is not immediately evident. Our mutagenicity studies[8,9], comparing two excision-defective (classical) XP strains with normal human cells, after exposure to ultraviolet radiation, have demonstrated that the increased frequencies are directly correlated with a lack of excision and that the excision process itself does not introduce mutations. They suggest that the critical event responsible for mutations is DNA replication on a template containing unexcised lesions. Although, for a particular dose of radiation, classical XP cells have a greater number of unexcised lesions remaining in their DNA than do normal cells, this should not be the case for cells derived from XP variants The latter, because they exhibit normal rates of excision[4-6], should contain the same number of unexcised lesions as normal cells at any particular time after irradiation. The higher mutation frequencies observed in the variants, however, could be caused by some process related to replication of DNA containing unexcised lesions Trivial explanations for the observed phenomenon—such as a difference in length of cell cycle in the two strains so that less time for excision before DNA replication was available to the variant cells, or a partial synchronisation of one strain but not the other—have been ruled out (data not shown).

Fig. 3 Relationship between frequency of ultraviolet light-induced mutations and its cytotoxic effect in normal human cells (●) and in variant strain, XP4BE (■). Data are taken from Figs 1 and 2 Assuming a random (Poisson) distribution of lethal events in the population, a survival of 37, 13.5 and 5% corresponds to an average number of lethal events per cell of 1, 2, and 3, respectively. The lines for each strain have been calculated by the method of least squares.



To investigate the question further, we carried out a series of alkaline sucrose gradient studies[8], similar to those reported by Lehmann *et al.*[6] to compare the size of newly synthesised DNA in XP variants with that of normal cells. The results of our sedimentation velocity studies on the size of DNA confirm their findings that in cells from XP variants, as compared with normal cells, there is some kind of defect in DNA synthesis after ultraviolet irradiation[8]. Lehmann has suggested that such DNA is synthesised from the 'inter-dimer' spaces on the irradiated DNA template and the size of the DNA is initially small because DNA synthesis is discontinuous at each dimer—that is, 'gaps' are left Within 1–2 h, normal cells restore the DNA to the size found in unirradiated controls, but XP variants take about four times longer to effect such lengthening of the DNA molecule. Nevertheless, since the newly synthesised low molecular weight DNA seems to attain the same size as that from unirradiated cells long before the next round of DNA synthesis (data not shown), it is not obvious how a mere difference in rate of 'gap filling'[15] or replication on a template which still contains unexcised dimers[16,17] could be responsible for the increased cytotoxic or mutagenic effects of ultraviolet irradiation observed in these strains Note, however, that the actual size of DNA in the cell is much larger than that which can be resolved with alkaline sucrose gradients (namely ~ 1.5×10^8 daltons (ref 6)). If the DNA in the variant cells does reach full size, one would have to assume that the slowness of the process is correlated with, or is a result of, some other defect which is ultimately responsible for both lethal and mutagenic events.

We examined the relationship between the frequency of mutations induced and the lethal effect of ultraviolet irradiation in the XP variant and compared it with that for the normal strain (data taken from Figs 1 and 2) to obtain the relationship shown in Fig. 3. The lack of overlapping points indicates that the variant actually receives a greater number of mutagenic events per lethal event than normal cells. Further work is under way to determine whether these two lines are the result of a fundamental biological difference between XP variants and normal cells, or whether the difference can be accounted for by problems inherent in comparing their different cell strains.

Nevertheless, our present results—showing that fibroblasts derived from skin biopsies from patients with greatly increased susceptibility to skin cancer caused by exposure to sunlight, exhibit frequencies of ultraviolet light-induced mutations significantly higher than normal—provide strong support for the somatic mutation hypothesis for the origin of cancer.

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Immunological identity of urokinase and ovarian carcinoma plasminogen activator released in tissue culture

THERE is evidence that fibrinolytic enzymes are involved in the growth of tumours. It has long been known that malignant tumours in culture lyse clotted plasma substrates[1–3]. Davidson *et al.*[4] found a giant-cell carcinoma of the lung to be rich in plasminogen activator and to produce it in tissue culture. Rifkin *et al.*[5] reported an abundant release of fibrinolytic enzymes by mammalian fibroblasts transformed by DNA or RNA viruses, by chemically or virally induced mammary carcinomas and by human malignant tumours in culture. Normal fibroblasts and cultures of other normal tissues released little or no fibrinolytic enzymes. Christman and Acs[6] found neoplastic cells, whether transformed by oncogenic viruses or by chemical agents, to release a plasminogen activator not released by normal cells. This activator was characterised as a serine protease with a molecular weight of approximately 50,000. The presence of fibrinolytic activity has been demonstrated in human ovarian tumours[7] as well as in ascitic fluid associated with such tumours[8]. We have studied a stable plasminogen-activating enzyme, released by ovarian carcinoma in tissue culture, and we report here the apparent identity of this plasminogen activator (TA) and the activator produced by foetal kidney and present in normal urine—that is, urokinase.

The properties of the TA were examined both directly in the culture medium and after purification by affinity chromatography on para-aminobenzamidine carbodiimide coupled to CH-Sepharose 4B (Fig. 1). TA was bound firmly enough in the column and only a barely detectable amount of activity was not absorbed. Most of the protein passed through the column. After washing until no A_{280} was detectable, elution by shift of *p*H resulted in a sharp peak of activity. Gel filtration on Sephadex G-100 of TA purified by affinity chromatography resulted in an incomplete separation of two active peaks with molecular weights of about 35,000 and 52,000, respectively.

Neutralisation and immunodiffusion experiments were carried out with rabbit antiserum raised against Leo urokinase (Leo Pharmaceutical, Denmark) purified by affinity chromatography and found to be monospecific[10]. Dilution series of purified TA were incubated with IgG from urokinase antiserum respectively with IgG from normal rabbit serum as a control (Table 1). The results demonstrate a distinct neutralisation of TA by the anti-urokinase IgG. Double diffusion in agarose gel between the concentrated culture media of six different ovarian cancers and anti-urokinase IgG resulted in distinct immunoprecipitation lines (Fig. 2). Furthermore, a complete immunological identity could be demonstrated between the

Table 1 Neutralisation of the activity of TA by anti-urokinase antibodies

Dilution	1 : 2	1 : 4	1 : 8	1 : 16
TA + normal IgG	15–18	11–14	9–10	4–5
TA + anti-urokinase IgG	Traces	0	0	0

Dilution series of purified TA incubated in proportion 2 : 1 with IgG prepared from rabbit urokinase antiserum as well as from normal rabbit serum. The fibrinolytic activity was tested on fibrin plates and expressed as the diameter of the lysed zones.

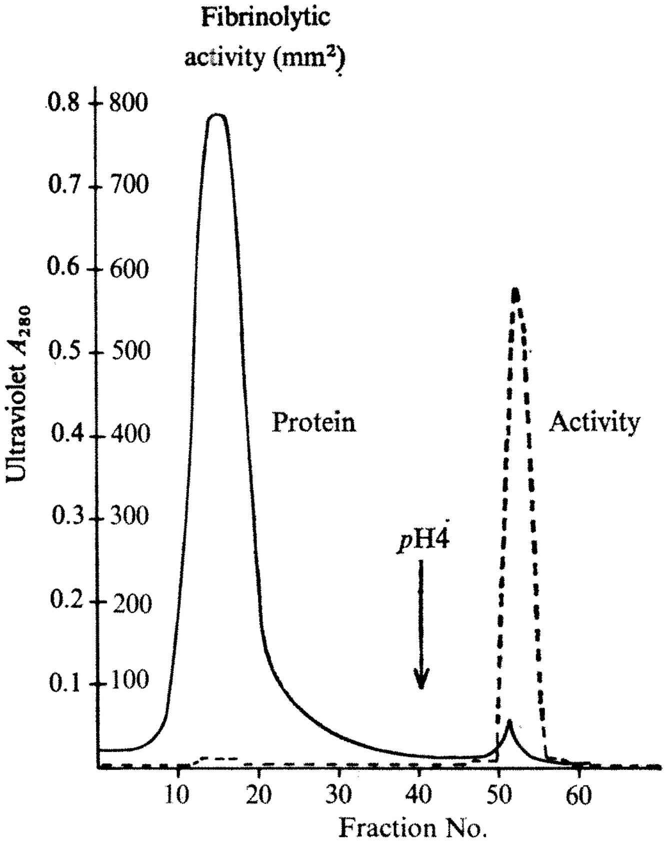


Fig. 1 Affinity chromatography of tumour culture medium on a 1.6 × 12-cm column of benzamidine–Sepharose; 150 mg *p*-aminobenzamidine (Sigma, USA) was coupled by water-soluble carbodiimide to 7.5 g CH-Sepharose 4B (Pharmacia Fine Chemicals, Uppsala). The gel was equilibrated with 0.1 M sodium phosphate buffer, *p*H 7.0 and 0.4 M NaCl. Before chromatography the tissue culture medium was centrifuged, filtered and dialysed against the same buffer; 20 ml dialysed culture medium was applied (flow rate 42 ml h^{-1}). Elution was carried out by changing the buffer to 0.1 M acetate, *p*H 4.0, and 0.4 M NaCl. Absorbance, 280 nm. The fibrinolytic activity was tested on plasminogen-containing fibrin plates and expressed as the diameter of the lysed zones. Specimens of ovarian papillary cystadenocarcinoma were obtained at laparotomy and processed for organ and cell culture. For organ culture the specimen was divided into small fragments of about 1 mm^3. These tumour explants were then placed on gelatin foam slices (Spongostan, Ferrosan, Sweden) in Petri dishes. A purely synthetic medium (Parker 199, SBL, Stockholm) was then added. Survival of the explants was checked by serial sectioning and histological examination. For cell culture the cells were prepared from the carcinoma specimen using the Borell net piston[9]. They were cultured as mixed monolayer cultures in Parker 199. The cultures were run in 5% CO_2 in air, and the medium was collected every third day and frozen. The culture media from the organ or cell cultures were constantly found to be fibrinolytically active when tested on plasminogen-containing human fibrin (Kabi, Stockholm) plates. Such activity was never observed on plasminogen-free bovine fibrin (Poviet Producten, The Netherlands) plates. When purified plasminogen was added to the plasminogen-free plates, lysis occurred, thereby showing the presence of a plasminogen activator. The highest fibrinolytic activity was found in medium of the organ cultures, which were thus the most suitable for obtaining the plasminogen-activating enzyme.

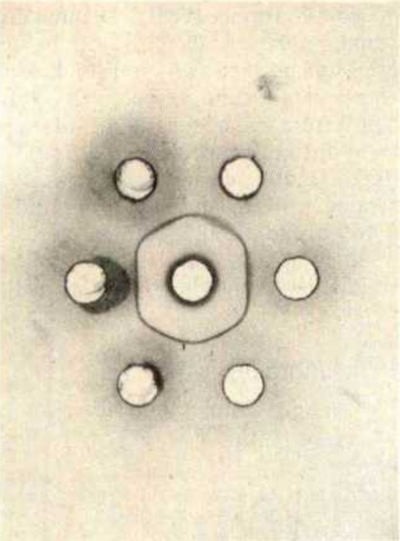


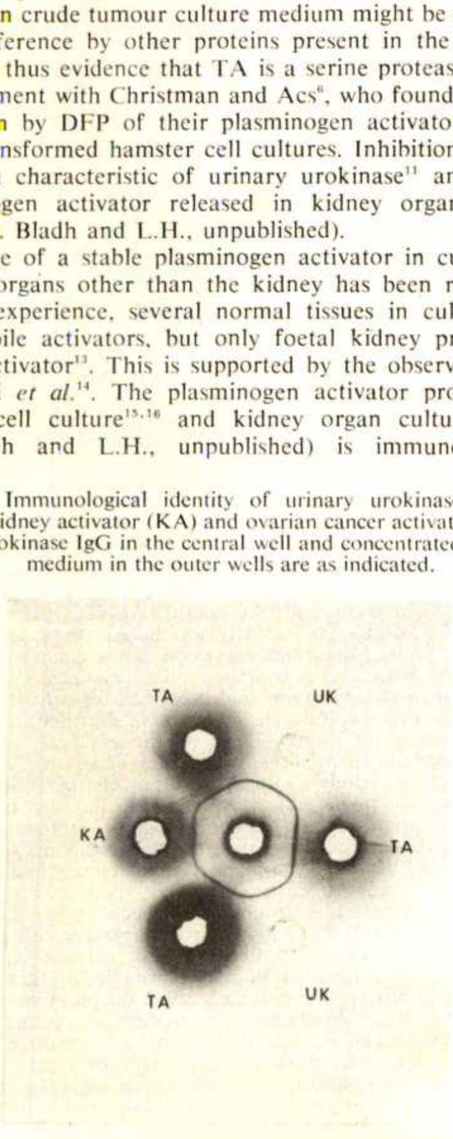
Fig. 2 Double diffusion of tumour culture medium against anti-urokinase IgG. The media from six different ovarian cancer cultures were concentrated 50–100 times on Minicon micro-concentrator cells (Amicon, The Netherlands) and placed in the outer wells and the antiserum was deposited in the central well.

tumour activator and both urinary urokinase and the activator produced by foetal kidney in culture (Fig. 3).

Figure 4 shows the inhibition of TA by di-isopropylfluorophosphate (DFP). The delayed inhibition of the activity in crude tumour culture medium might be explained by interference by other proteins present in the medium. There is thus evidence that TA is a serine protease. This is in agreement with Christman and Acs[6], who found a similar inhibition by DFP of their plasminogen activator isolated from transformed hamster cell cultures. Inhibition by DFP is also a characteristic of urinary urokinase[11] and of the plasminogen activator released in kidney organ culture (B.A., B. Bladh and L.H., unpublished).

Release of a stable plasminogen activator in cultures of normal organs other than the kidney has been reported[12]. In our experience, several normal tissues in culture produce labile activators, but only foetal kidney produces a stable activator[13]. This is supported by the observations by Kucinski *et al.*[14]. The plasminogen activator produced in kidney cell culture[15,16] and kidney organ culture (B.A., B. Bladh and L.H., unpublished) is immunologically identical to urokinase isolated from urine. We have now shown that the plasminogen activator produced by ovarian carcinoma cells is immunologically identical to urinary urokinase as well as the activator released in kidney organ or cell culture. The molecular weight distribution of TA on gel filtration suggests two molecular forms. It is known that urokinase exists in two molecular forms with molecular weights of about 31,000 and 54,000 (refs 17 and 18).

Fig. 3 Immunological identity of urinary urokinase (UK), foetal kidney activator (KA) and ovarian cancer activator (TA). Anti-urokinase IgG in the central well and concentrated culture medium in the outer wells are as indicated.



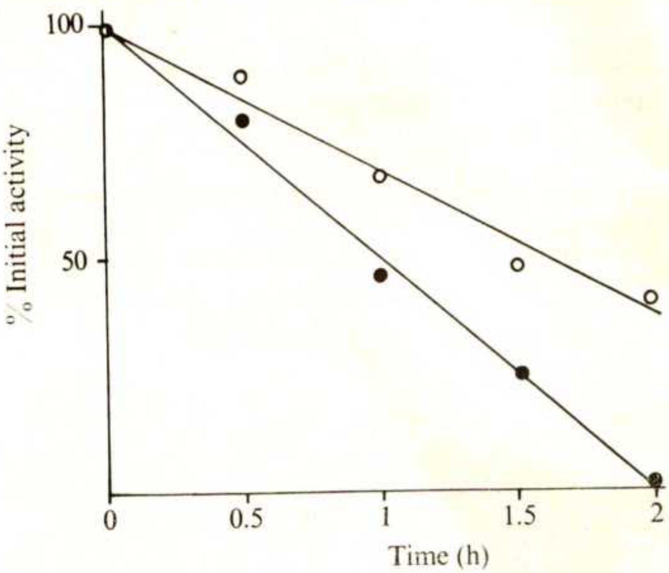


Fig. 4 Inhibition of TA by DFP. Tumour culture medium (○) and TA purified by affinity chromatography (●) were incubated at room temperature with equal amounts of DFP at a final concentration of 2.5×10^{-3} M in the reaction mixture (stock solution of 10^{-2} M DFP in propylene glycol diluted in 0.05 M Tris buffer, *p*H 7.8) for 0, 0.5, 1, 1.5 and 2 h. After these incubation times non-reacted DFP was removed by gel filtration on small columns filled with Sephadex G25 (Pharmacia, Uppsala) calibrated with dextran blue and K_2CrO_4. The fractions present in the void volumes were then tested for fibrinolytic activity on fibrin plates containing plasminogen. The activity of the fractions was measured as the diameter of the lysed zone. The diameter varies linearly with the logarithm of the enzyme concentration[20]. The activity is expressed as a percentage of initial activity.

From the data presented it can be inferred that the TA produced by ovarian carcinoma cells is very similar to, if not identical with, urokinase. In patients with malignant ovarian tumours, fibrinogen–fibrin degradation products in the blood are a common finding[19], and extremely large amounts are constantly found in ascitic fluid[8]. This indicates that the TA demonstrated here is highly active *in vivo* in such patients. The role played by the TA in the survival, growth and spread of ovarian cancer can be investigated further in experimental models with urokinase antibodies.

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Hormone-like role of H–Y antigen in bovine freemartin gonad

In cattle, chorionic vascular anastomosis with a male twin modifies sexual development of a female twin foetus[1,2]. In this condition, testosterone-dependent masculinisation of the genital tract is only moderate: the ovary is virilised to resemble a small testis in the extreme case (for reviews see refs 3 and 4). Since testosterone derived from the male twin cannot modify the ovary[5], the cellular theory of freemartinism has been proposed[6,7]. This theory is based on the finding that freemartins are XY/XX chimaeras with regard to not only their haemopoietic organs[8,9] but also their gonads[9]. Indeed, the XY/XX mosaic or chimaeric gonad of mammals seems to show the definite tendency to develop as a testis. For example, so-called XX human males may actually be cryptic XY/XX mosaics[10], and in certain strain combinations, a great preponderance of males is seen among the experimentally produced XY/XX mosaic mice[11]. It has been proposed that H–Y antigen disseminated by XY gonadal cells entices neighbouring XX cells to engage in testicular organisation[12]. Here we report the confirmation of this proposal on the strongly virilised foetal freemartin gonad.

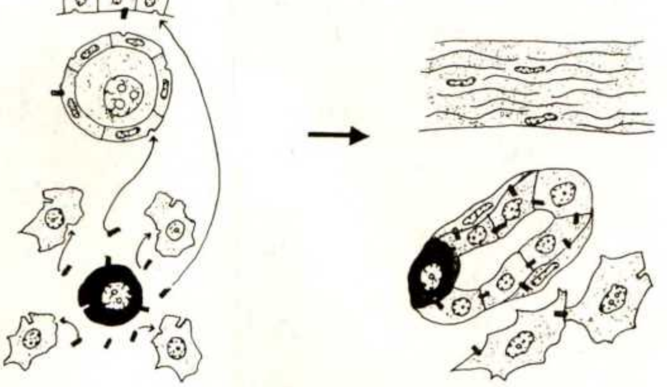

Fig. 1 Proposed virilisation mechanism of bovine freemartin gonad. Host XX germ cell and somatic elements are shaded; a donor XY cell is shown in black. We suggest that XX gonadal cells, although not possessing H–Y antigen, are equipped with specific receptor sites as well as anchorage sites for H–Y antigen (indentations on the cell surface). When coated with disseminated H–Y antigen, XX gonadal cells tend to organise testicular structure. On the left is a freemartin gonad at about 50 d of gestation: a donor XY cell that has remained quiescent allowing ovarian organisation to proceed, has just started to disseminate H–Y antigen (solid black rods). When coated with H–Y antigen, XX cells of the organised ovarian cortex are destroyed. On the right is a strongly virilised freemartin gonad at 150–175 d of gestation (Fig. 2): The ovarian cortex is replaced by a layer of hard connective tissue which now constitutes the thick tunica albuginea of the testis-like gonad. XX gonadal cells of the ovarian medulla, now coated by H–Y antigen, are forming a seminiferous tubule-like structure together with donor XY cell. Two XX cells are differentiating into Leydig cells. As most XX germ cells that were in the ovarian cortex have been destroyed, a paucity of primordial germ cells characterises the testis-like freemartin gonad (Fig. 2).

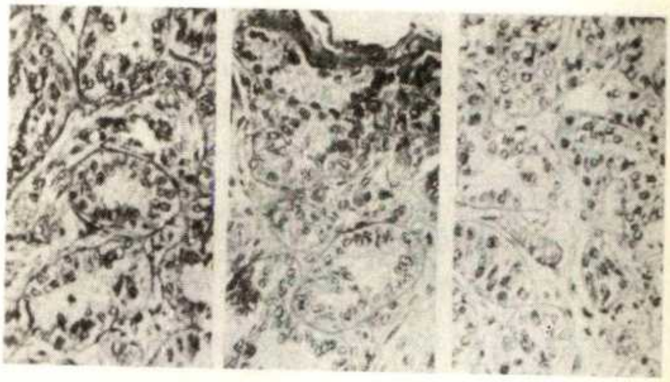

Fig. 2 Photomicrographs of histological sections from gonadal fragments of three freemartin foetuses. Lenses used were 45 × 6. From left to right, crown–rump lengths of foetuses were 40, 44 and 48 cm. Testis-like architecture is obvious in all three. Gonads from 48-cm freemartin foetus (right frame) were used in serological tests shown in Fig. 3 and Table 1.

Because viable cells recognise and interact with each other through their plasma membranes, cell surface components must have a key role during organogenesis[13,14]. For this reason the phylogenetically conserved plasma membrane component recognised serologically as H–Y antigen may be the long-sought product of the testis-determining gene[15]. Indeed H–Y antigen is specified by a Y-chromosomal gene[16], and all our studies support the proposed identity between H–Y antigen and the testis-determining gene product. Thus, we have invariably observed an association between the presence of H–Y antigen and testicular differentiation in both mice and men, regardless of phenotype[17].

We propose here that XY cells from the bull twin disseminate H–Y antigen, thus coating the majority of host XX cells. H–Y antigen-coated host XX cells, together with donor XY cells, could thereby subvert development of the freemartin gonad, causing it to differentiate as a testis. According to this scheme (Fig. 1), H–Y antigen would be an enticement that XY cells extend to neighbouring XX cells. Alternatively, each H–Y antigen-bearing XY cell might come in contact with a succession of XX cells, instructing them to engage in testicular organisation. In this scheme

Table 1 Mixed MHA.HA test showing reaction of mouse H–Y antiserum with mouse sperm after absorption with cells from the gonads of foetal bovine normal female, freemartin female and male twin

	H–Y antiserum aliquot		
Unabs.	Abs. ♀	Abs. ♂ twin	Abs. ♀ twin†
33.0*	28.5	19.0	12.0

To avoid repeated centrifugation, MHA.HA tests were performed by centrifuging sperm through a discontinuous density gradient containing wash layer and reagents in sequence[16,28]. Gradients were established in narrow tubes by adding foetal bovine serum to each layer in a concentration adjusted to prevent intermixing. Equal volumes (0.05 ml) of a suspension containing 5–10 × 10^6 BALB epididymal sperm per ml and of H–Y antiserum were reacted. The "sensitised" sperm were then placed in the gradient tube and centrifuged slowly through a wash layer and a layer containing sheep red blood cells (SRBC) sensitised with a rabbit synthetic hybrid antibody of the specificity: anti-mouse Ig/Anti-SRBC. Sperm which bound H–Y antibody (and therefore also the hybrid antibody) bound SRBC to form rosettes. Absorption of H–Y antibody in the MHA.HA test was indicated by a fall in the number of rosettes compared with the number counted after reaction of sperm with unabsorbed H–Y antiserum. MHA.HA tests were scored as coded samples by an observer who recorded the counts of rosettes and free sperm in a haemocytometer field. The values (percentages) shown above were derived from the formula: number of rosettes / number of rosettes + free sperm. Any sperm cell to which three or more SRBC had absorbed was scored as a rosette. Details of this procedure are given in ref. 16.

* Number of rosettes per 100 sperm cells.

† See Fig. 2, right frame, for cross section of freemartin gonad used in this test.

the H–Y antigen does not need to be transferred from XY cells to XX cells.

XY/XX chimaerism has always been more conspicuous in the gonad of the foetal or newborn male twin than in the gonad of the foetal or newborn freemartin. But XX cells do not feminise the testis although they may indirectly cause a marked reduction in the reproductive fitness of the bull twin[18]. On the other hand, the proportion of XY mitotic figures sampled from foetal freemartin gonads seldom exceeds 10%; newborn freemartin gonads often show no trace of XY cells[7,9]. Nevertheless XX gonads containing only a minority of XY cells may be extremely virilised as noted above. It follows that observation of a substantial amount of H–Y antigen in strongly virilised freemartin gonads would go beyond confirmation of chimaerism, and would suggest that a few XY programmed effector cells can disseminate enough H–Y antigen (or act otherwise) to influence the development of host XX cells.

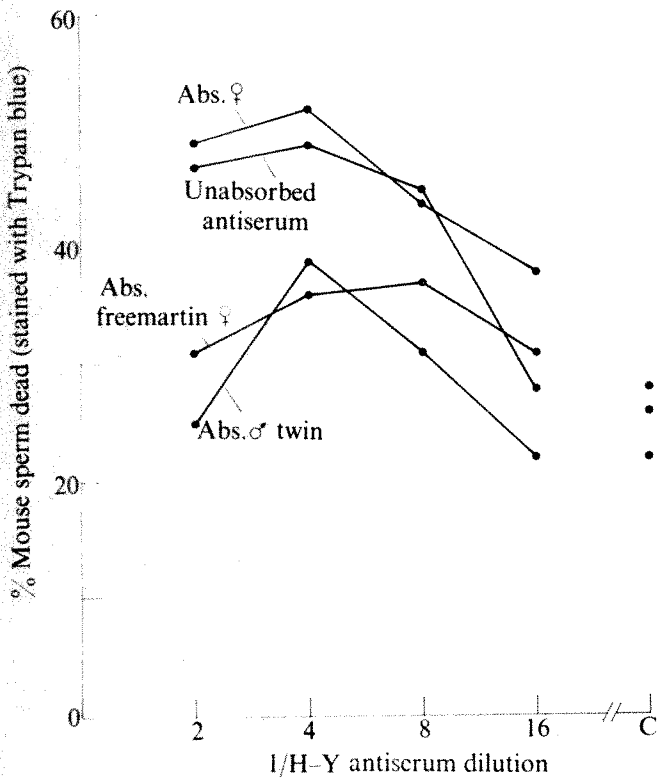

Fig. 3 Cytotoxicity test on mouse sperm with mouse H–Y antiserum after absorption with gonadal cells from foetal bovine female, freemartin and male twin. Abs. denotes absorption with cells from the subject indicated. C denotes control (complement included, antiserum omitted). H–Y antisera were raised in B6 female mice by weekly intraperitoneal injections of spleen cells from B6 males. Absorption was accomplished by suspending foetal gonad cells in small tubes containing 0.15 ml of H–Y antiserum diluted one half (one part cells : five parts antiserum) and allowing the suspension to stand for 30 min on ice. After absorption the tubes were centrifuged and each supernatant (antiserum) was removed and divided into aliquots for use in both cytotoxicity and mixed haemadsorption–hybrid antibody (MHA.HA) tests. The cytotoxicity test was performed with BALB epididymal sperm according to the method of Goldberg *et al.*[27]. Equal volumes (0.05 ml) of H–Y antiserum (serially diluted 1/2 to 1/16), sperm (5×10^6 per ml), and absorbed rabbit serum (complement source) diluted 1/15, were incubated at 37 °C for 50 min. Trypan blue dye (0.1 ml) was added during the last 10 min of incubation to stain dead sperm, and the suspensions were read as coded samples in a haemocytometer. The decrease in cytotoxic titre shown after absorption with freemartin and male twin gonadal cells indicates that these cells contain H–Y antigen. H–Y antisera used in this test were also used in the MHA.HA test shown in Table 1. A cross section of a fragment from the foetal freemartin gonad used in this test is shown in the right frame of Fig. 2. Details of the sperm cytotoxicity test are given in ref. 16.

After ascertaining that normal bovine foetal ovaries do not express H–Y antigen whereas H–Y antigen is prominent in bovine foetal testes, we collected freemartin foetuses of 40–48 cm crown–rump length (150–175 d of gestation, when the freemartin ovarian medulla undergoes masculine reorganisation[19,20]). Three freemartin foetuses were chosen for serological determination of H–Y antigen expression, because histological examination of fragments from their gonads revealed strongly virilised testis-like architecture (Fig. 2). The results of our cytotoxicity and mixed haemadsorption–hybrid antibody tests on one of them are shown as examples in Fig. 3 and Table 1. Gonads from all these freemartins typed unequivocally and equally H–Y+.

In avian species, the heterogametic (ZW) female sex possesses W-linked histocompatibility antigen[21], and homology has been demonstrated between H–W antigen and mammalian H–Y antigen[22]. According to our hypothesis, a ZW/ZZ mosaic gonad of avian species should be organised into an ovary. This is true for chimaeric heterosexual twins of chicken and duck[23]: the testis of a male twin is modified to resemble an ovary. Avian primordial germ cells utilise the vascular route for their migration[24]. Thus, gonadal chimaerism is a certainty in heterosexual twins.

It should be remembered that certain constraints are apparently imposed on a hormone-like action of H–Y antigen in many mammalian species. For example, in marmoset monkeys, the apparently mosaic gonad of chimaeric female twins develops into a functional ovary[25]. Even in the mouse, a great preponderance of males among the XY/XX mosaics occurs only in certain strain combinations[11]. Maybe XY gonadal cells of the marmoset-type mammalian species as well as of some strains of the mouse do not disseminate enough H–Y antigen. Conversely, the complex locus for major histocompatibility (MHC) antigens might be a principal source of the contraints. H–Y antigen seem to coexist with MHC antigens on the plasma membrane[26]. Perhaps in the marmoset-type species a genetic difference at the MHC locus precludes effective transfer of H–Y antigen from XY to XX cells. The same might be true for some strain combinations of the mouse. Even in cattle, freemartin gonads exhibit divergent degrees of virilisation. These are problems for future studies.

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Induction of specific proteins in hyphae of *Achlya ambisexualis* by the steroid hormone antheridiol

SEXUAL differentiation in the male and female strains of the water mould *Achlya ambisexualis* occurs through a series of stages mediated by steroid hormones[1]. The species-specific and sex-specific hormones, antheridiol and oogoniol[2], initiate the development of the male and female sex organs, respectively. Because antheridiol induction depends on RNA and protein synthesis[3] and steroid hormones are thought to influence the synthesis of specific proteins in the target cells[4], we have investigated the pattern of proteins synthesised in the male strain of *A. ambisexualis* after administration of antheridiol. We found an 'induced protein' with a molecular weight of 69,000. It was detectable 1 h after addition of the hormone. This is analogous to the effect of oestradiol on protein synthesis in the uterus of immature rats[5,6].

Initial experiments were carried out with total unlabelled protein obtained from hyphae treated for 3 h with antheridiol. Antheridia were clearly recognisable in these hyphae. After extraction and solubilisation of proteins, they were resolved on a sodium dodecyl sulphate (SDS)–polyacrylamide gel and stained. The pattern of proteins was scanned at 600 nm and recorded (Fig. 1*a*). This pattern did not differ significantly from that of the proteins obtained from uninduced hyphae (Fig. 1*b*). Pre-existing proteins probably mask the proteins synthesised in response to antheridiol, which are only a small percentage of the total protein present.

Double label experiments were done to detect proteins synthesised when antheridiol was administered. *A. ambisexualis* was treated with antheridiol for various times and the proteins synthesised at different stages of induction were pulse labelled with ^{3}H-leucine. After solubilisation of hyphal proteins, ^{3}H-labelled proteins were mixed with ^{14}C-leucine-pulse-labelled proteins obtained by the same process from uninduced control hyphae. This mixture of proteins containing ^{3}H-leucine and ^{14}C-leucine was then electrophoresed on SDS–polyacrylamide gels and the patterns of radioactivity were determined.

This procedure was carried out with ^{3}H-leucine-labelled proteins synthesised in a 20-min pulse after addition of antheridiol to hyphae for 0, 0.5, 1, 2, 3, 5 and 7 h and ^{14}C-leucine-labelled proteins from uninduced mycelium. The gels were sliced and the ^{3}H and ^{14}C radioactivity in each fraction was determined. To minimise statistical variation, the ratio of ^{3}H to ^{14}C in each gel slice was standardised by summing the individual ratios of a gel, calculating the mean of the individual fractions and dividing each ratio by the mean ratio. The deviations of the individual standardised ratios [$(R_i/\bar{R})-1$] were calculated and plotted (Fig. 2) as a function of the gel slice number as described by Caroll *et al.*[7]. (R_i, individual slice ratio, $\bar{R}$, $= R_i/n$, the mean ratio, $R_i/\bar{R}$ the standardised ratio.) The last twenty slices containing only a small fraction of the labelled proteins were not included because ^{14}C counts were too low to give a reliable ratio.

No appreciable deviation from $(R_i/\bar{R})-1 = 0$ was observed in Fig. 2*a* This mixture of uninduced proteins labelled with ^{14}C-leucine and ^{3}H-leucine for 20 min served as a control for labelling, extraction and counting. The pattern observed in mycelium induced for 30 min (Fig. 2*b*) showed no single induced protein although larger proteins seemed to be synthesised preferentially

One hour after addition of antheridiol (Fig. 2*c*), a predominant peak of protein synthesised in induced mycelium

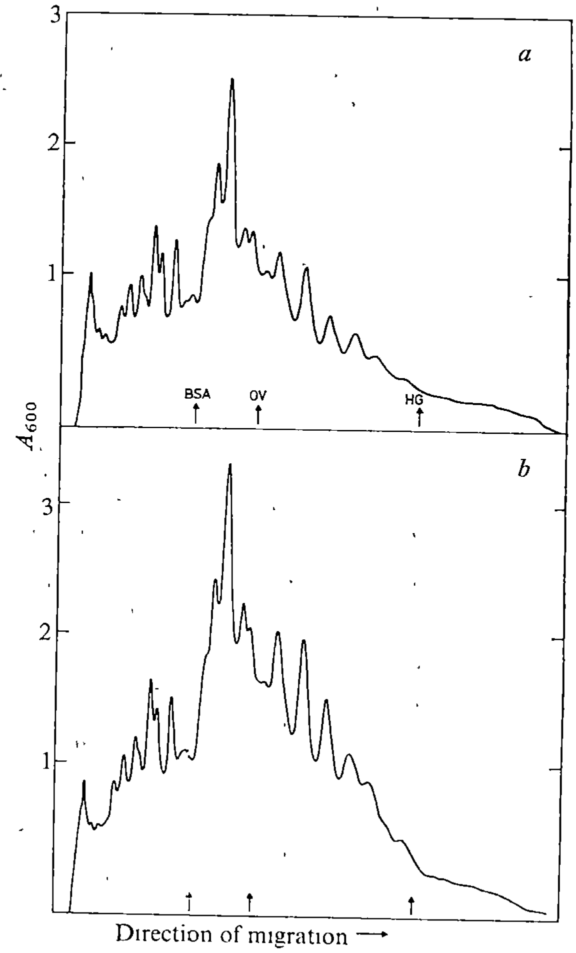

Fig. 1 SDS–polyacrylamide gel electrophoresis of total protein extracted from *A. ambisexualis* after 3 h of treatment with antheridiol (*a*) and from uninduced control mycelium (*b*). Cultures of *A. ambisexualis* E87 were maintained on agar plates of production medium[11]. A plug of 3 mm diameter was cut out of the agar plate and incubated in 4 ml for 2 d at 25 °C. Partially purified antheridiol solution (from Dr A. Barksdale, New York Botanical Garden, 50 µl, 0.4 mg ml^{-1}, 30,000 U ml^{-1} in 25% methanol[12,13]) was then added to the radially growing mycelium Three hours after addition of antheridiol, growth was stopped by chilling, mycelium was collected by centrifugation for 10 min at 10,000*g* and washed twice with cold distilled water. The mycelium was then resuspended in 10 ml of 50 mM Na phosphate buffer, *p*H 7.2, and homogenised in a Willems–Polytron homogeniser at setting 7 for 2 min at 2 °C. SDS was added to 2% and the homogenate was boiled for 1 min. After removal of insoluble material by centrifugation for 10 min at 10,000*g*, the proteins were precipitated with 0.1 volume of 50% trichloroacetic acid washed with 5 ml of ethanol, ethanol–ether (1 : 1) and ether, dried and redissolved in 10 mM Na phosphate containing 2% SDS. Electrophoresis was then carried out on 7.5% SDS–polyacrylamide gels for 4.5 h and 10 mA per gel[14]. Gels were stained with Coomassie blue, destained electrophoretically and scanned at 600 nm with a Gilford gel scanner. A parallel gel contained the molecular weight markers bovine serum albumin (68,000), ovalbumin (43,000) and haemoglobin (15,500).

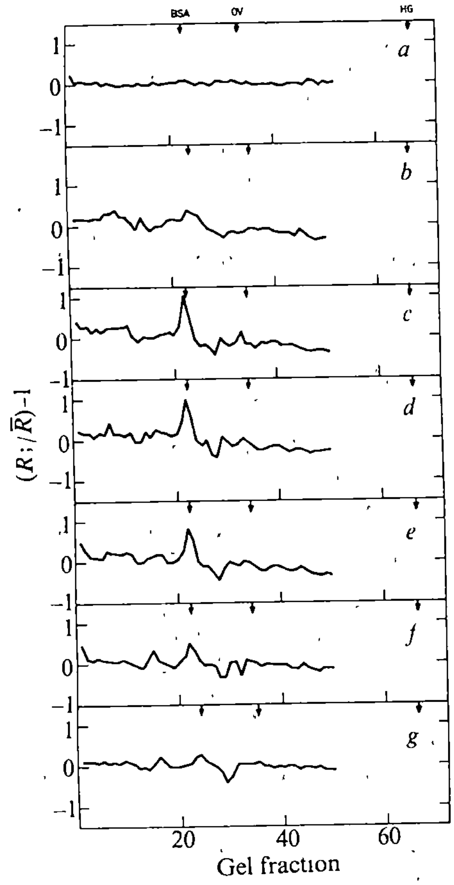

Fig. 2 SDS-gel electrophoresis of proteins extracted from total mycelium after 20 min of labelling with L-^{3}H-leucine (50 μCi ml^{-1} 54 Ci mmol^{-1}). Labelling was preceded by treatment with antheridiol for 0(*a*); 0.5(*b*); 1(*c*); 2(*d*); 3(*e*), 5(*f*) and 7 h(*g*) The mycelium was cultured and induced with antheridiol, the proteins were extracted as described in Fig 1 and more than 95% of the labelled proteins were solubilised. The ^{3}H-labelled proteins were mixed with ^{14}C-leucine-labelled proteins at a ratio of ^{3}H to ^{14}C radioactivity of approximately 5 The ^{14}C-leucine-labelled proteins were obtained from an uninduced culture labelled for 20 min with L-^{14}C-leucine (1.25 μCi ml^{-1}, 324 mCi mmol^{-1}). Electrophoresis was carried out as described for Fig 1. Gels were sliced and ^{3}H and ^{14}C radioactivity in each fraction was determined by liquid scintillation counting in 0.5 ml of NCS solubiliser and 10 ml of toluene-based scintillation fluid. The ^{3}H and ^{14}C counts obtained simultaneously from each fraction were used to calculate the ratios as described in the text. The standardised ratios were calculated to minimise statistical variation. The three marker proteins bovine serum albumin (BSA), ovalbumin (OV) and haemoglobin (HG) were run in a parallel gel. They are indicated by arrows.

became apparent This protein had a molecular weight of approximately 69,000 and could be recognised clearly in the gel pattern of newly synthesised proteins (Fig. 3*a*). Comparison with the proteins in control hyphae (Fig. 3*b*) revealed a pronounced shift in the pattern of protein synthesis towards the 69,000 molecular weight protein. This protein also accounted for the main difference in protein synthesis between induced and uninduced mycelium 2 and 3 h after addition of hormone (Fig. 2*d* and *e*). The most abundant protein (or proteins) migrating at about 55,000 molecular weight (observed in Fig. 1, which represents the steady state population of proteins, as well as in Fig. 3*b* where pulse labelled proteins are shown) was synthesised to a lesser extent in induced mycelium. This reduction in synthesis was reflected in the decreased $(R_i/\bar{R})-1$ at about fraction 28

When the time of treatment with antheridiol was increased to 5 and 7 h (Fig. 2*f* and *g*) before the 20-min pulse labelling, the synthesis of the 69,000 molecular weight protein was reduced. The pattern of proteins synthesised 7 h after induction resembled that of the uninduced mycelium There was still decreased production of the protein(s) migrating at about 55,000 molecular weight, however. The decrease in synthesis of the 69,000 molecular weight protein after 7 h was not due to exhaustion of antheridiol in the medium since this medium still had the biological activity to induce antheridia when added to previously uninduced mycelium.

Electron microscopic and enzymatic studies[8,9] have suggested that the enzyme cellulase, concentrated in vesicles at the site of formation of antheridia and responsible for localised cell wall softening, is released into the culture medium. This led us to identify the proteins which are excreted into the culture medium after induction. Figure 4 shows the radioactive proteins that can be found in the medium after hormone induction for 0, 0.5, 1, 3 and 7 h followed by labelling for 20 min with ^{3}H-leucine. These proteins comprise approximately 1% of total labelled proteins. Uninduced mycelium (Fig. 4*a*) shows a broad distribution of labelled, excreted proteins.

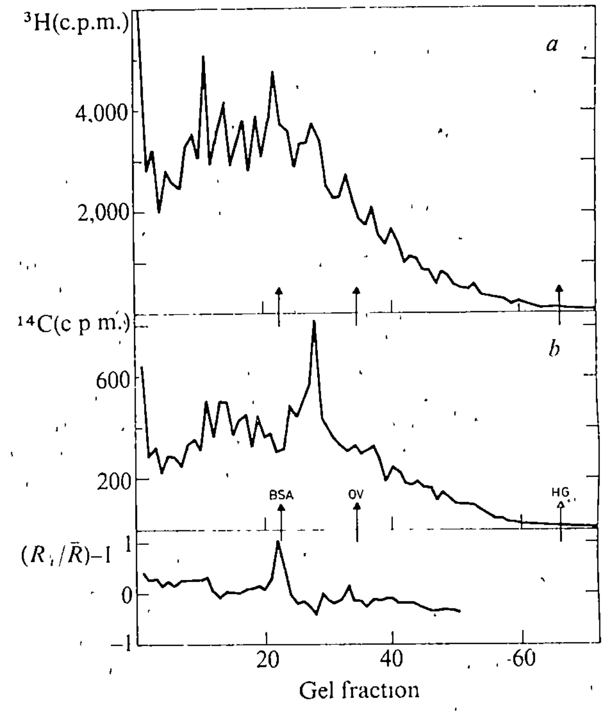

Fig. 3 SDS-polyacrylamide gel electrophoresis of proteins extracted from mycelium labelled for 20 min after 1 h of treatment with antheridiol as described for Fig. 2*c*. The ^{3}H-labelled proteins obtained from induced mycelium (*a*) and the ^{14}C-labelled proteins obtained from the uninduced control mycelium (*b*) were coelectrophoresed and the radioactivity patterns obtained simultaneously were plotted on separate panels The standardised ratios were calculated as described in the text.

Mycelium induced for 0.5 and 1 h showed a predominant peak of excreted protein migrating at 40,000 molecular weight (Fig. 4*b* and *c*). In addition to this protein, three peaks of excreted protein migrating at 29,000, 64,000 and 88,000 molecular weight were observed after 1 h. Increasing the labelling time to 3 and 7 h (Fig. 4*d* and *e*) resulted in excretion of a complex pattern of proteins, different from that found inside the mycelium and therefore not attributable to cell lysis.

Although proteins made by *A. ambisexualis* in response to antheridiol have not been isolated and identified, this report shows that the addition of antheridiol results in the synthesis of specific proteins. The labelling protocol in these experiments (a 20-min pulse label) restricts the analysis to those proteins which are synthesised most rapidly. A protein of molecular weight 69,000 is made preferentially during the first few hours of induction. It is first synthesised 0.5–1 h after addition of hormone and precedes the microscopic appearance of antheridial initials[10] which can be observed about 1 h later. After 3 h the fraction of the total protein synthesis which this protein comprises decreases again. This protein was not detected in the culture medium, but because of the labelling procedure used

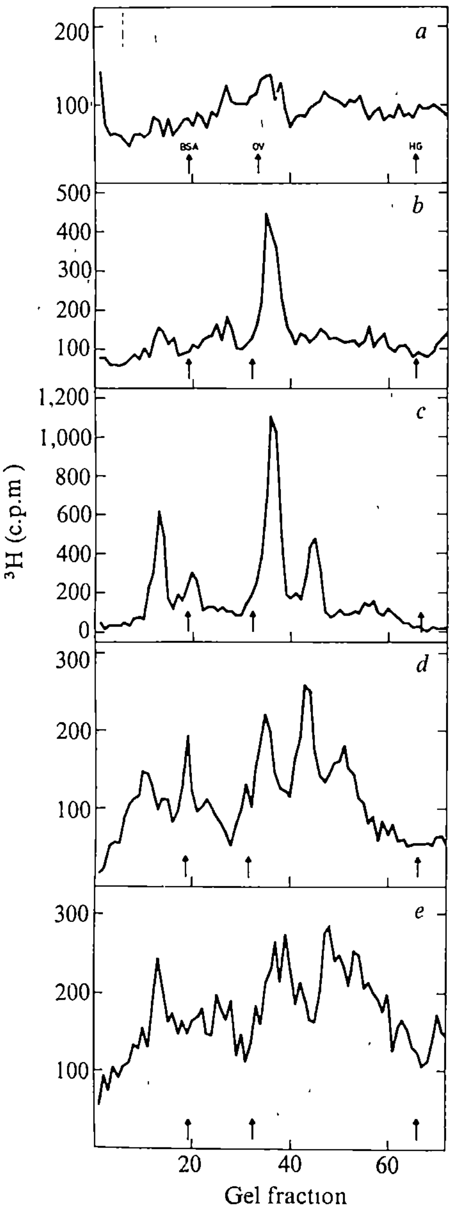

Fig. 4 SDS-polyacrylamide gel electrophoresis of proteins excreted by mycelium treated with antheridiol for 0(*a*); 0.5(*b*), 1(*c*); 3(*d*) and 7(*e*) h and then labelled for 20 min with L-4,-5-^{3}H-leucine (50 μCi ml^{-1}, 54 Ci mmol^{-1}) as described in Fig. 2. After removal of the mycelium from the culture medium by centrifugation for 10 min at 10,000*g*, the supernatant was centrifuged again at 10,000*g* for 10 min. The second supernatant was mixed with 0.1 volume of 50% cold trichloroacetic acid and the precipitated proteins were washed with ethanol, ethanol-ether (1 : 1) and ether. The washed proteins were resuspended in 10 mM Na phosphate, *p*H 7.2, and 2% SDS, electrophoresed and counted as described for Figs 1 and 2.

only proteins synthesised and exported rapidly would be found. This protein could have a role similar to that of the 'induced protein' found in immature rat uterus after induction by oestradiol.

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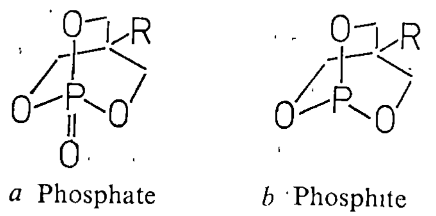
Bicyclic phosphorus esters that are potent convulsants and GABA antagonists

A SERIES of bicyclic organophosphorus (PTBO) compounds (Fig. 1) described by Bellet and Casida[1] were found to produce seizures and death within minutes of their injection into mice. In spite of the chemical resemblance of these substances to anticholinesterase agents, doses of the PTBO compounds many times those necessary to produce death had no effect on brain cholinesterase activity[1], and the mechanism of their convulsant action remained obscure. We have, therefore, investigated the action of ethyl, isopropyl and pentyl PTBO compounds as putative antagonists both of the depressant action of γ-aminobutyrate (GABA) on single neurones in the rat brain and of the depolarising action of GABA on the isolated rat superior cervical ganglion. Our results suggest that the convulsant action of the PTBO compounds may be related to their ability to antagonise the actions of GABA released as an inhibitory neurotransmitter[2] at synapses in the brain.



a Phosphate *b* Phosphite

Fig. 1 Structure of bicyclic phosphorus esters. *a*, 4-(R)-1 phospha 2, 6, 7-trioxabicyclo(2, 2, 2)octane-1-oxide ((R)PTBO); *b*, 4-(R)-1 phospha 2,6,7-trioxabicyclo(2,2,2)octane The compounds investigated had R = isopropyl (IPTBO), R = ethyl (EPTBO and ethyl phosphite) and R = pentyl (PPTBO).

The compounds were synthesised according to the method of Wadsworth and Emmons[3], and then tested for convulsant activity in mice. They were also compared with the known GABA antagonists bicuculline[4] and picrotoxin[5]. The isopropyl compound (IPTBO) was the most potent of the PTBO series and was approximately equipotent with bicuculline on a molar basis. The ethyl phosphate compound (EPTBO) had one-tenth of the potency of IPTBO and was approximately equipotent with picrotoxin whereas the pentyl compound (PPTBO) and the ethyl phosphite had less than one-hundredth the potency of IPTBO. Our estimates of the potency of IPTBO and EPTBO were in close agreement with those of Bellet and Casida[1]. All six substances produced the same pattern of convulsions consisting of rapid clonic jerks, leading to tonic extension with larger doses. The similarity of the gross actions of all these convulsants suggested that it would be worth while to examine the bicyclic phosphorus esters for GABA antagonist activity, particularly as IPTBO was as potent a convulsant as bicuculline

The central actions of the PTBO compounds were investigated in urethane anaesthetised adult rats, using techniques for single neurone recording and microiontophoretic application of drugs as described previously[6]. All single neurone records were extracellular and taken from neurones in the medulla, some of which were physiologically identified as belonging to the cuneate nucleus[7]. In our first series of experiments attempts were made to apply EPTBO or IPTBO by microiontophoresis from seven-barrelled micropipettes. Solutions (10 mM) were prepared in distilled water, both with and without 150 mM NaCl and both positive and negative expelling currents up to 200 nA were used. No discernible effect was seen on the activity of 10 neurones studied or on the responses of these neurones to the depressant actions of GABA and glycine. It would not be predicted that these compounds would be ionised in solution and we therefore concluded that it would not be possible to expel them or control their diffusional efflux. Accordingly, a second series of experiments was started in which GABA was administered by microiontophoresis and the PTBO compounds were injected intravenously. Most of these experiments were performed using IPTBO, the most potent convulsant, and in 8 out of 11 trials attenuation of the depressant action of GABA was seen with subsequent recovery. An example of an experiment of this type is shown in Fig. 2. EPTBO (4 trials) and PPTBO (2 trials) also attenuated the action of GABA when given intravenously, although much higher doses (that is, in the ratio of their convulsant potencies) had to be given. No evidence of antagonism was seen with any of these substances until they were given in a dose sufficient to produce EEG signs of convulsive activity, although these doses were smaller than those needed to produce myoclonus.

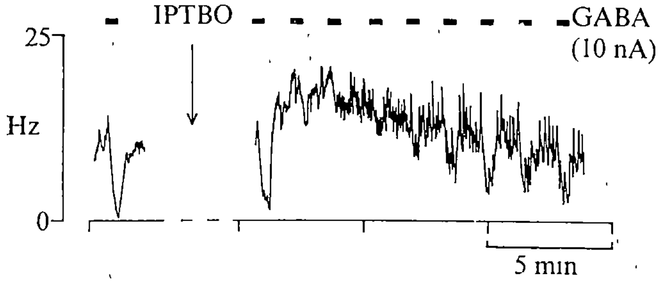


Fig. 2 Analogue rate meter recording of the spontaneous firing of a single neurone in the rat medulla (vertical scale firing rate in Hz, horizontal scale time, 5 min per division). GABA was applied microiontophoretically with a current of 10 nA for 30 s at regular intervals of 60 s determined by a digitimer. At the arrow 40 µg (2×10^{-7} mol) IPTBO was injected into one jugular vein and applications of GABA following this injection were seen to be less effective. Within 10 min of the injection a substantial recovery was seen and this was a consistent observation in other similar experiments Note the increase in burst firing by this neurone after IPTBO (shown by a less regular ratemeter trace) which coincided with the start of ictal spiking in the EEG. Action potential size was monitored during this experiment and remained effectively constant, suggesting that this result was not attributable to relative movement of the micropipette tip and the neurone No myoclonus or cardiovascular correlates of seizures were seen.

In six experiments glycine was applied alternately with GABA and intravenous IPTBO was then found to attenuate the depressant action of both amino acids, although glycine seemed to be less affected than GABA on each occasion. It is therefore possible to conclude that IPTBO is a consistent but not a selective GABA antagonist when administered intravenously.

Where microiontophoresis is not practicable, more controllable experiments on putative GABA antagonists can be performed *in vitro* in a preparation such as the isolated superior cervical ganglion of the rat. GABA depolarises ganglionic neurones by an action at receptors comparable in both ligand specificity and ionic dependence to receptors for GABA on central neurones[8,9]. This action of GABA in ganglia can be readily detected as a change in potential[8-10]. Ganglia were excised from Wistar rats under urethane anaesthesia, desheathed and suspended vertically between the two non-depolarisable $Ag^+/AgCl$ electrodes placed in contact with the postganglionic trunk and ganglion body. The tissue was superfused at 1 ml min^{-1} with Krebs' solution containing hyoscine (2 6 µM) at 25 °C and the potential difference across the electrodes monitored on a Servoscribe 1s recorder.

The addition of any of the four PTBO derivatives to the Krebs' solution superfusing the ganglion antagonised the depolarising action of GABA. The effect was dose dependent. Mean log dose–responses curves for GABA from five ganglia obtained in the absence and presence of 5.2, 52 and 156 µm IPTBO are shown in Fig. 3. The presence of IPTBO shifted the curve to the right, a greater shift occurring with increase in IPTBO concentration.

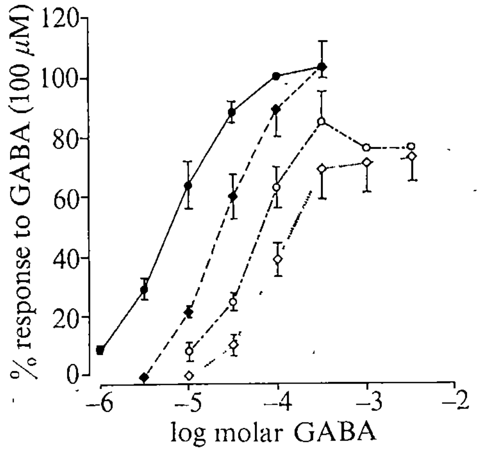


Fig. 3 Effect of IPTBO on ganglionic depolarisation produced by GABA. Ordinate: mean peak depolarisations produced during 1-min applications of GABA plotted as a percentage of the response to 100 µM GABA in the same experiment (100 µM GABA = 100%). Abscissa: log molar concentration of GABA. Responses were obtained in the absence (●) or presence of IPTBO at concentrations of 5.2 µM (◆), 52 µM (○) and 156 µM (◇). The data were obtained from five separate ganglia. Vertical bars represent s e.m. GABA was applied at 20 min intervals and each concentration of IPTBO was in contact with the tissue at least 15 min before subsequent applications of GABA.

Depression of the maximum response to GABA occurred at 52 and 156 µm IPTBO, an effect which contrasts with that of bicuculline and bicuculline methochloride but compares with that of tetramethylenedisulphotetramine (TETS)[8,9,11]. The effect of IPTBO was, however, readily reversible and, within 15 min of its removal, responses equal to control level could be obtained. IPTBO seemed to be comparable in potency with (+)-bicuculline methochloride and TETS. Equimolar amounts of the three substances depressed responses to GABA (30 µM) by approximately the same amount. The similar potencies of (+)-bicuculline methochloride and TETS in the ganglion have already been noted[11]. The relative potencies of the compounds can be summarised as. IPTBO=1, EPTBO=0 1, ethyl phosphite=0.03, PPTBO<0.01. These results are in close agreement with the ratio of their convulsant potencies.

The compounds seemed to be specific antagonists of the action of GABA in the ganglion. Responses to the cholinomimetic, carbachol, which also depolarises ganglia in a dose-dependent manner[12], were unaffected by the PTBO compounds when responses to GABA were blocked or considerably reduced and the action of the other ganglionic stimulants, 5-hydroxytryptamine and angiotensin, was similarly unaffected by the presence of IPTBO.

The results obtained with the compounds examined so far suggest that the potency of these compounds as GABA antagonists depends solely on the alkyl group (R) in the molecule (Fig. 1). Increasing the size of this group from ethyl to isopropyl produces a substantial increase in potency both as a convulsant and as a GABA antagonist but a further increase in size to pentyl produces a dramatic decrease in potency. It would therefore seem that these compounds combine with a site on the subsynaptic membrane that has a lipophilic cleft of a defined size into which the alkyl group fits. The molecule can be considered as a conformationally restricted skeleton maintaining the alkyl group and the rest of the molecule in the correct three-dimensional relationship. It is interesting to note that ethyl phosphite (Fig 1) was active both *in vivo* and *in vitro* as it has been suggested that this compound is only active after bio-oxidation to the phosphate[2]. The ethyl phosphite, although hygroscopic, is stable to water[13] and the observation that it is of lower potency than the corresponding phosphate may give a pointer to possible chemical substitution at the phosphorus-containing part of the molecule. Further synthesis of compounds in this group is in progress to allow a more confident interpretation of the structure-activity relationships involved This is an important area of research as EPTBO, in sufficient concentration to convulse rats[14], has been shown to be present in the smoke given off by burning fire-retarded polyurethane foams and the problem of finding an antidote to poisoning by compounds such as these can only be solved by a complete understanding of their mode of action.

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Independence of β-adrenergic and thyrotropin receptors linked to adenylate cyclase in the thyroid

CATECHOLAMINES stimulate thyroid function in several species, including cattle, man and mouse (brief review in ref. 1). As with the pituitary-derived tropic hormone, thyrotropin, an initial step in this process seems to be stimulation of adenylate cyclase associated with the thyroid cell plasma membrane, leading to increased intracellular adenosine-3′-5′ cyclic monophosphate (cyclic AMP). The response to catecholamines is mediated by a β-adrenergic receptor[2,3] and we have examined whether, for a given membrane preparation, the response of adenylate cyclase to the β-adrenergic agonist isoproterenol can be varied independently of the response to thyrotropin. Our results indicate that the two receptor–adenylate cyclase systems function separately.

Bovine thyroid membranes were prepared as before[4], and adenylate cyclase activity was determined in the conditions described previously[5]. The cyclic AMP formed was assayed as before[6]. The methods of Smith and Hall[7] were used to prepare plasma membranes from the human thyroid, and to study the binding of iodinated thyrotropin.

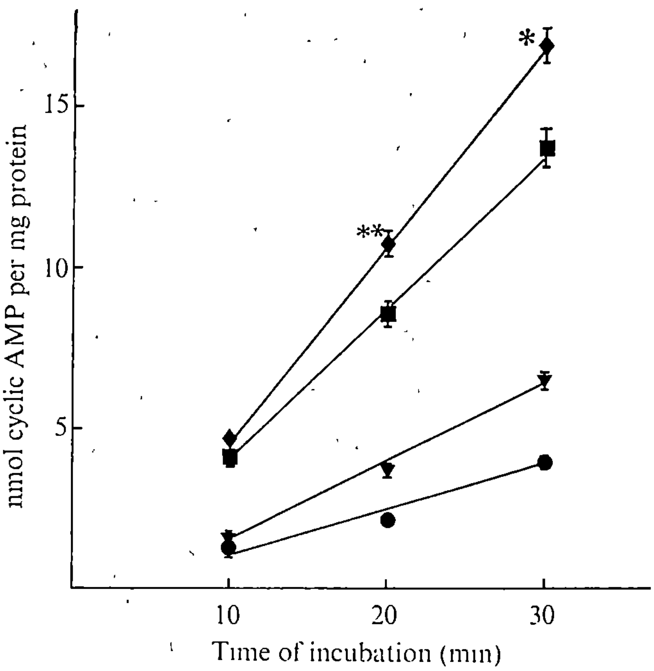

Fig. 1 Time course of stimulation of adenylate cyclase in bovine thyroid plasma membranes by isoproterenol (▼, 2×10^{-5} M), thyrotropin (■, 50 mU ml^{-1}) and a combination of the two (diamonds) Membrane preparations (10 µg membrane protein) were added to tubes equilibrated at 37 °C, containing 2.5 mM ATP, 5 mM $MgCl_2$, 10 mM theophylline, 25 mM Tris-HCl, *p*H 7 4, 10 mM creatine phosphate, 20 µg of crystalline rabbit muscle creatinine kinase. Bovine thyrotropin (Thytropar, Armour) and isoproterenol were added as indicated, in a final incubation volume of 120 µl Results are expressed as means of quadruplicate determinations of adenylate cyclase activity ± s.e.m., each assayed for cyclic AMP in duplicate Isoproterenol plus thyrotropin compared with thyrotropin alone: * $P<0.05$; ** $P<0.005$ ●, Basal level of cyclic AMP.

Figure 1 shows a time course of basal adenylate cyclase activity stimulated by thyrotropin and isoproterenol in bovine thyroid preparations. Significant stimulation by thyrotropin (50 mU ml^{-1}) and isoproterenol (2×10^{-5} M) was seen after 10 and 20 min of incubation, respectively. Simultaneous addition of both stimulators resulted in further stimulation of adenylate cyclase activity; caused in an additive manner. Because 2×10^{-5} M isoproterenol is sufficient to provoke a maximal response (Fig. 2 and ref. 3), the increased adenylate cyclase activity in the presence of thyrotropin suggests stimulation through separate systems. We examined this possibility by testing for selective inhibition of the response to the two stimulators.

l-Propranolol and polyphloretin phosphate (PPP) were used as selective inhibitors of isoproterenol and thyrotropin stimulation respectively. l-Propranolol inhibits both isoproterenol and thyrotropin stimulation of adenylate cyclase activity in bovine thyroid membrane preparations[3,5], but the concentration required to inhibit the former is several orders of magnitude lower than that needed to inhibit the

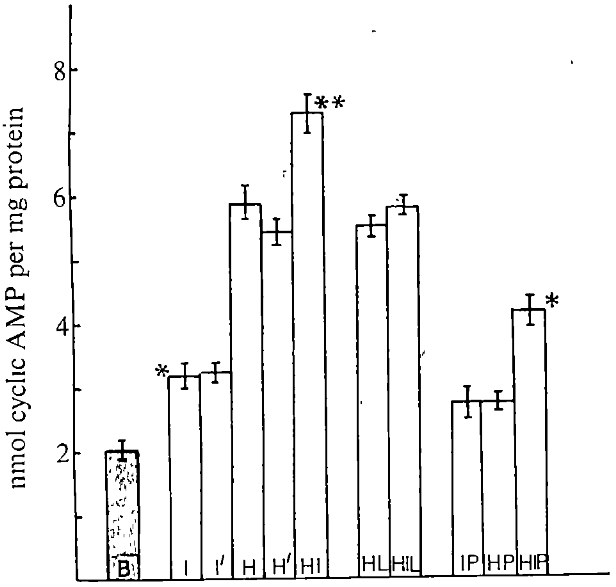

Fig. 2 Selective inhibition of isoproterenol and thyrotropin stimulation of adenylate cyclase in bovine thyroid plasma membranes. Conditions of incubation as for Fig 1. Incubation was for 30 min and the membrane preparation used was not that used for the experiment shown in Fig. 1 Results expressed as mean of quadruplicate determinations of adenylate cyclase activity ± s.e.m., each assayed for cyclic AMP in duplicate The hatched column shows the basal level. I, 2 × 10^{-5} M isoproterenol; I^1, 10^{-4} M isoproterenol; H, thyrotropin (50 mU ml^{-1}); H^1, thyrotropin (100 mU ml^{-1}); L, 10^{-6} M L-propranolol; P, PPP (10 µg ml^{-1}).

*I compared with basal, and HIP compared with HP, $P < 0.0005$. †HI compared with H, $P <$ 0 0005, IP not significantly different from I

latter. Conversely, PPP (10 µg ml^{-1}) inhibits stimulation by thyrotropin in bovine thyroid plasma membranes, but not that by isoproterenol (Fig. 2). As Fig. 2 shows, adenylate cyclase activity stimulated by the presence of both thyrotropin and isoproterenol was reduced by addition of l-propranolol (10^{-6} M) to the activity seen in the presence of thyrotropin alone. Confirming previous results, the inhibitor was without significant effect on stimulation due to thyrotropin alone. PPP (10 µg ml^{-1}) selectively reduced stimulation by thyrotropin, however, such that in the presence of both thyrotropin and isoproterenol, the response seemed to be that due to isoproterenol plus the diminished response to thyrotropin. Such selective inhibition was consistent with independence of the receptor–adenylate cyclase systems.

Table 1 Failure of isoproterenol or adrenaline to displace thyrotropin bound to human thyroid membranes

Additions	% Iodinated thyrotropin bound
None	20 3±0 81
+Thyrotropin (165 mU ml^{-1})	3 7±0 31
+IgG*	11 0±1 0
+NIgG*	20.6±1.7
+Isoproterenol (10^{-6} M)	19 5±0 72
(10^{-5} M)	20 1±0 74
(10^{-4} M)	21.1±1.26
+Adrenaline (10^{-4} M)	22.6±1 35

Incubation conditions were as described in ref. 7, except that label was added at the beginning of the 1 h of incubation at 37 °C simultaneously with unlabelled thyrotropin, isoproterenol or adrenaline. Final concentrations in the incubation tube are shown, and membranes were present at a concentration of 400 µg membrane protein ml^{-1} Results are expressed as the mean of duplicate determinations ± s d

*Immunoglobulin G (IgG) preparation from thyrotoxic serum (×5 concentrate) No inhibition observed with a similar preparation of IgG from control serum (NIgG)

Table 1 confirms reports that iodinated thyrotropin binds to membrane preparations from the human thyroid in a reversible manner, and can be inhibited by an immunoglobulin fraction from thyrotoxic sera[7–10]. Moreover, the specific nature of binding was confirmed by the observation that neither isoproterenol, present in concentrations ranging from 10^{-6} to 10^{-4} M, nor 10^{-4} M adrenaline caused significant displacement of bound thyrotropin. This suggests that the receptor system for isoproterenol and thyrotropin are functionally disassociated in thyroid membrane preparations.

Thus, although Spaulding and Burrow[2] failed to detect additivity when studying thyrotropin and isoproterenol stimulation of intracellular cyclic AMP in slices of thyroid tissue, our results indicate that in plasma membrane preparations, receptors for thyrotropin linked to adenylate cyclase function independently from the β-adrenergic receptors.

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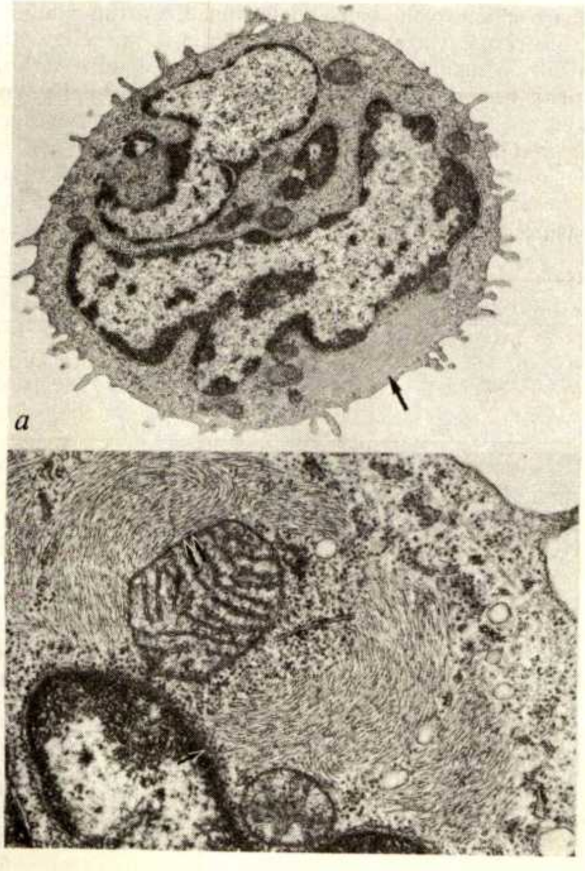
Different distribution pattern of 100-Å filaments in resting and locomotive leukaemia cells

Two types of filaments have been described in the cytoplasm of non-muscular cells—microfilaments (40–70 Å in diameter) and 100-Å filaments (90–110 Å). Microfilaments have been found in almost every type of animal cell[1], and they are known to be actin-like[2] and believed to have a role in many contractile processes[1,3]. In contrast, the 100-Å filaments have been observed only in a limited number of cell types, such as tissue culture cells[2,4], smooth[5] and skeletal[6] muscle cells, nerve cells[7,8] and tumour cells[9,10], and are thought not to be actin-like[2,3,10,11]. The function of 100-Å filaments is not clear although they have been suggested to act in the intracellular transport of organelles in fibroblasts[4], the axoplasmatic transport in nerve cells[12,13] and the spreading of cells[3], or to serve as a cytoskeleton[14]. Induction of 100-Å filaments by colcemide[6] and cytochalasin B and colcemide[15,16] has also been demonstrated. A clue to their function may lie in the demonstration, reported here, that the distribution of the 100-Å filaments differs in resting and locomotive cells.

We are studying the correlation between motility and ultrastructure in leukaemia cells Our main experimental model is the undifferentiated transplantable leukaemia L 5222 (ref 17), propagated in the inbred BD-IX rat[18]. By time-lapse cinematography, the cells of this leukaemia are recorded in two functional states[19], as resting cells which are completely spherical, and as locomotive cells which have a conspicuous extension at the rear end resembling the uropod of stimulated lymphocytes[20]. This difference in shape makes it possible to distinguish between cells fixed at rest and in locomotion, respectively. For electron microscopy, cells were processed by double fixation (2% glutaraldehyde, 1% osmium tetroxide), ethanol dehydration, and embedding in Epon. To determine the actin- or non-actin-like nature of the filaments, L 5222 cells were glycerinated and incubated with HMM according to the procedure of Ishikawa *et al.*[2], and then prepared for electron microscopy. Thin sections of normal and HMM-treated cells were photographed with a Siemens 102 electron microscope.

L 5222 cells have an appreciable amount of both microfilaments and 100-Å filaments. The microfilaments bind HMM by forming characteristic arrowhead complexes, which proves their actin-like nature. On the other hand, 100-Å filaments show no reaction with HMM. Microfilaments are arranged in a fine mat-like structure beneath the cell membrane. This pattern is identical in resting and in locomotive cells. In contrast, there is a striking difference in the distribution patterns of the 100-Å filaments in the two functional states of L 5222 cells. Resting cells have thick bundles consisting of 100-Å filaments in parallel array (Fig. 1*a*), closely resembling the aggregates of 100-Å filaments recorded in cultures of myogenic and fibrogenic chick embryo cells[6,16]. The large bundles can reach a diameter of several μm, and the cytoplasmic rim between cell membrane and nucleus may be filled out by them. The 100-Å filaments seem to be associated closely with mitochondria and nuclear membrane (Fig. 1*b*). In locomotive cells, there are no large bundles, and the 100-Å filaments are distributed singly and in small groups within the uropod-like protrusion (Fig. 2).

Fig. 1 *a*, Resting L 5222 leukaemia cell with a large bundle of 100-Å filaments (arrow) (×10,875). *b*, Close association of a bundle with mitochondria (double arrow) and nuclear membrane (arrow) (×25,230).



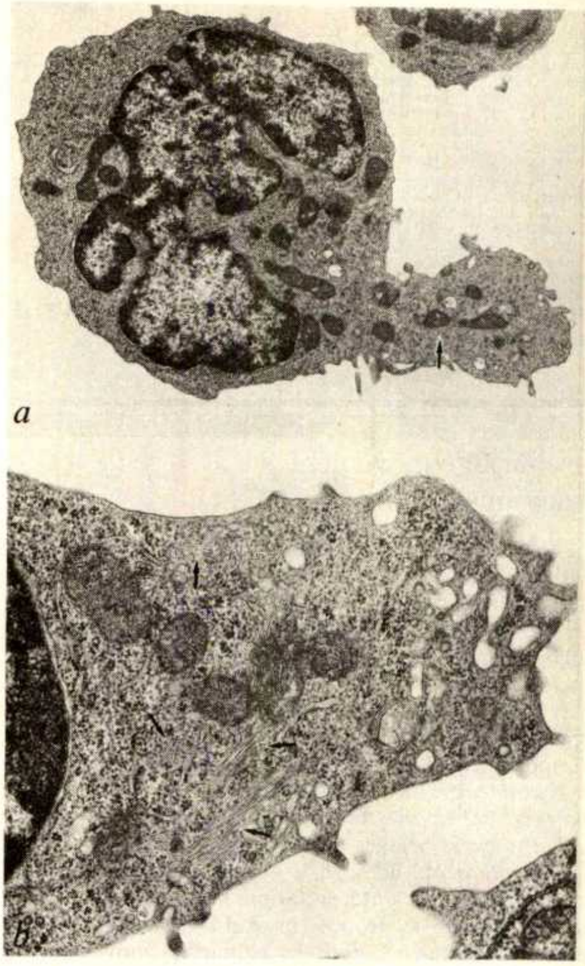


Fig. 2 *a*, Locomotive L 5222 cell with uropod-like protrusion (×7,680). *b*, Higher magnification of another uropod-like protrusion (×24,000). Small groups of 100-Å filaments are indicated by arrows.

In preliminary studies of the motility of human leukaemia cells, we have found a similar correlation between the arrangement of 100-Å filaments and locomotive behaviour (unpublished results). In one case of an acute myeloid leukaemia, the lack of any cellular locomotion was associated with the occurrence of huge bundles of 100-Å filaments in nuclear indentations, whereas in the highly locomotive cells of another case of human leukaemia, the 100-Å filaments were found only in small groups.

In summary, it seems that in resting leukaemia cells the non-actin-like 100-Å filaments can be arranged in large bundles, whereas with the onset of locomotion these filaments become rather loosely distributed in the cytoplasm. The significance of the different distribution pattern of 100-Å filaments in resting and locomotive leukaemia cells is unclear and needs further experimental investigation.

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Cross-bridge movement during muscle contraction

WHEN vertebrate striated muscle contracts at constant length, the 1,0 reflection decreases in intensity while the 1,1 reflection increases in intensity[1,12]. These changes have been interpreted as arising from the movement of the cross bridges from the vicinity of the thick filaments in relaxed muscle to the vicinity of the thin filaments in the contracting state, and this movement has been thought to involve both radial and azimuthal components[2,3]. Lymn[4] has claimed that models with cross bridges near the thick filament do not simulate the intensity distribution of the higher order equatorial reflections of relaxed muscle, while models in which the cross bridges are extended to near the radius of the thin filaments do give good simulations. This has led him to contest the interpretation of the intensity changes given above, and to propose instead a model in which these changes are brought about by azimuthal movement alone. In this article we challenge Lymn's interpretation on several grounds.

Lymn based his conclusions on a study of five models. When we recalculated the intensities expected from his models, we noticed several discrepancies (Table 1). In his model I, Lymn's published value for the intensity of the 1,1 reflection is too high by almost a factor of two, and more importantly, in his model II, his value for the intensity of the 2,2 reflection is ten times too low. We have verified our computations where they differ from Lymn's by independently resynthesising the original structures by Fourier inversion. Lymn had rejected his model II (and all models in which the cross bridges are near the thick filament) in preference to his models IV and V (where the cross bridges are at higher radius) because he considered that his calculated intensities of the higher orders for model II were too low to account for the pattern from resting muscle. But when our calculated intensities are compared with those observed experimentally (Table 1) it is not easy to decide between models II, IV and V as representing resting muscle, and certainly model II can no longer be dismissed. By rotating the cross bridges in Lymn's model II through 12° and extending them by only 1 nm, we generated a model VI with short cross-bridge arms (Fig. 1) for which the agreement between observed and predicted intensities is considerably better than for any of Lymn's models with cross bridges at higher radius (Table 1). We believe, however, that all these models are somewhat unrealistic because the distribution of mass between the cross bridges, the backbone of the thick filament and the thin filament does not correspond to the values determined experimentally for vertebrate striated muscle[5,6]. Furthermore, we believe that detailed modelling experiments are premature at the moment because too little information is available concerning the number, shape and size of the cross bridges and their orientation relative to the thin filaments.

We have therefore attempted to derive a model of the equatorial density distribution directly from the measured intensities of the first five reflections by Fourier synthesis.

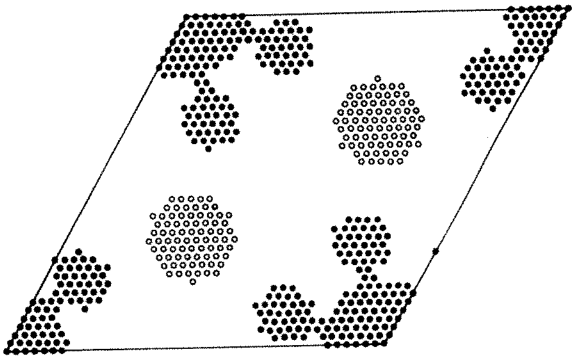

Fig. 1 Our model VI for the equatorial electron density distribution of a relaxed muscle. The model is based on Lymn's model II[4] with the thick filament rotated by 12° and the cross bridges extended by 1 nm. The spacing between adjacent points is 1 nm and the length of a side of the unit cell is 40 nm. ●, Electron density of 20 arbitrary units; ○, 15 such units; the remainder of the cell is considered to have zero density.

Table 1 Relative equatorial intensities

Model	I*	II	III	IV	V	VI	
Length of cross bridges	Long	Short	Short	Long	Long	Short	Experimental
Reflection							
1,0	100	100	100	100	100	100	100
1,1	190 (324)	47	33	30	26	39	44
2,0	47	7	3	36	4	7	9
2,1 1,2	62	2	11	12	20 (36)	9	9
3,0	16	0	18	155	35	4	12
2,2	34	21 (2)	1	11	19	10	20
3,1 1,3	30	0	1	23	85	19	
$I_{1,0}/I_{0,0}$	0.112	0.515	0.525	0.124	0.081	0.434	—

To avoid sampling errors intensity was calculated directly as

$$I_{hk} = (F_{hk})^2/(h^2+k^2+hk)^{1/2}$$

where

$$F_{hk} = \Sigma_x\Sigma_y\rho_{xy}\,\mathrm{exp}\eta[-2\pi i(hx+ky)]$$

and where h and k are the indices of the reflection and x and y are the fractional cell coordinates of each density point. I_{hk} was calculated for all points in the reciprocal lattice and the values for those points which superimpose on the pattern were then added. Values where Lymn's quoted intensities are significantly different from ours are underlined with Lymn's values given in parentheses. We have ignored errors of phases.

The intensity of the 0,0 reflection was calculated directly as

$$I_{0,0} = (\Sigma_x\Sigma_y\rho_{xy})^2$$

*Models I to V are described in Lymn[4] and model VI is shown in Fig. 1.

(For consistency, we have taken Lymn's reported values given in Table 1.) We have taken the phases of the 1,0 and 1,1 reflections as positive[3]. Of the possible combinations of + or − for the remaining reflections, adequate densities for the thin filaments were only obtained when the 3,0 reflection was positive. The best simulation of the relative masses of the thick and thin filaments was obtained when the 2,0, the 2,1 and the 1,2 reflections were all negative, although here the effect of phase was not so marked. The synthesis obtained is shown in Fig. 2 and is clearly in accord with models in which the cross bridges are at a radius less than that of the thin filaments. Similar results, but with lower relative thin filament densities, were obtained with other combinations of phases for the 2,0, the 2,1 and the 1,2 reflections. In these syntheses an implicit assumption of sixfold rotational symmetry has been made.

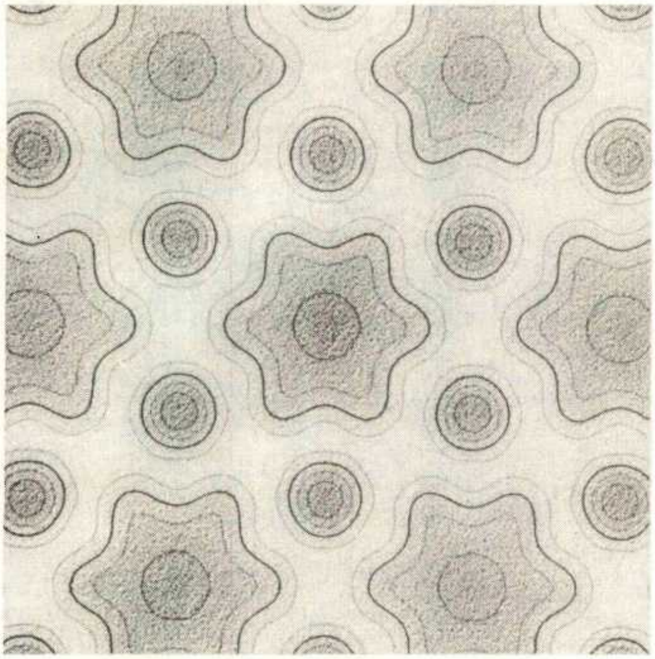

Fig. 2 A possible electron density distribution in relaxed vertebrate striated muscle at rest length calculated by a Fourier synthesis based on the intensities of the first five equatorial reflections as given by Lymn[4]. For simplicity, we have assumed that reflections which lie at the same distance from the origin (and thus contribute to one observed reflection) all have the same intensity. All combinations of positive and negative phases were tried for the 2,0, 2,1, 1,2 and 3,0 reflections and for this map the phases were −,−,− and + respectively. The four contours shown are equally spaced and the density is also simulated by stippling. Because only the first five reflections were included, the synthesis is not sharp, but it is consistent with the cross bridges being in an area approximately bounded by the second lowest contour (shown in heavy outline) and so being located at a radius less than that of the thin filaments, although their exact outline is unclear.

We feel that this synthesis should be treated with some caution until the symmetry of the thick filament has been established (which will show whether the phases are all real), although it is unlikely that the radial distribution of mass about the thick filament will be much different if the equatorial projection of the thick filament does have ninefold symmetry[7].

But whatever its exact relation to the distribution of density in relaxed muscle, this synthesis shows that it is possible to produce a model with the cross bridges at a radius less than that of the thin filaments which simulates the observed intensities exactly. We therefore believe that Lymn's rejection of any substantial radial movement was premature because his fundamental premise—that the equatorial pattern of relaxed muscle could not adequately be simulated with models with their cross bridges near the thick filaments—was incorrect.

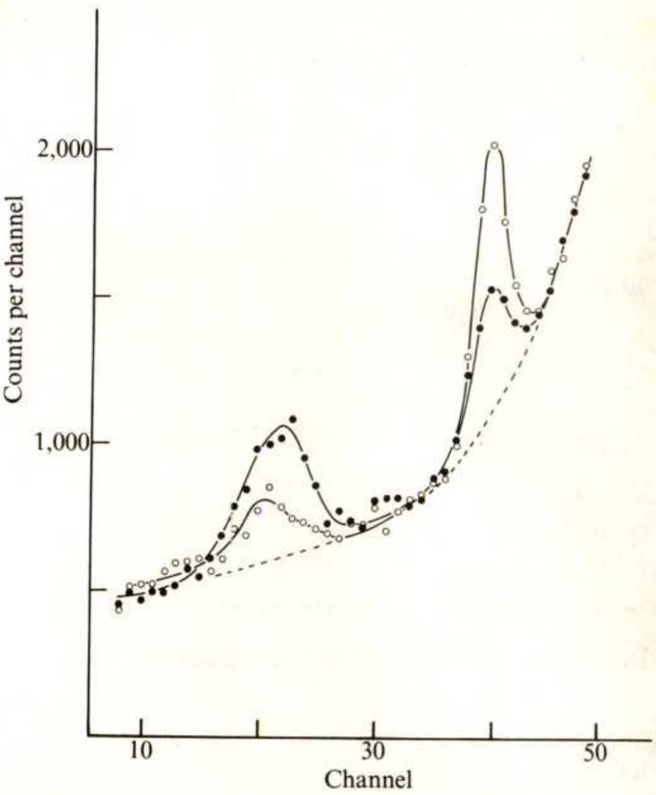

Fig. 3 Trace taken using a position sensitive counter[8,9] of the first two equatorial reflections from a frog sartorious muscle at rest length. ○, Relaxed; ●, the same muscle contracting isometrically. The total exposure was 10 s, which was summed from several short tetani.

We have also studied the absolute change in intensity of each of the first two equatorial reflections when a muscle contracts. The intensity of the 1,0 reflection is 50% (±10%, s.d. on 12 muscles) weaker in muscles during isometric contraction than in resting muscle (Fig. 3), while the intensity of the 1,1 reflection is 100% (±25%, s.d. on nine muscles) stronger. Thus the intensity ratio $I_{1,1}/I_{1,0}$ in contracting muscle is about 1.6 compared with 0.4 in relaxed muscle at rest length[1-10]. While it may be possible to explain the change in this ratio simply by azimuthal movement of the cross bridges, it is difficult to account for the large decrease in the absolute intensity of the 1,0 reflection by these means alone. For example, in Lymn's models, when the absolute change in the 1,0 reflection is calculated on going from his models IV or V (relaxed, long cross bridges) to model I (contracting) by comparing the intensity of these reflections with the 0,0 reflection, the 1,0 decreases by less than 10% and does not account for the experimentally observed change: models with more than six cross bridges in projection would predict an even smaller change. Moreover, Lymn's models represent movement of all the cross bridges: there is evidence[3,11] that suggests that not all of the cross bridges are attached to the thin filaments during contraction, and in this case, the changes predicted by Lymn's models IV and V would be even lower.

By rejecting Lymn's model we do not, of course, deny that there must be an azimuthal component to the cross-bridge movement; it is difficult to see how muscle could function efficiently without some azimuthal movement. On the evidence of our approximate calculations, however, it seems necessary to have a component of radial motion to account for the observed X-ray changes, and indeed such movement would also be necessary to allow the muscle to function at the different interfilament spacings found at different sarcomere lengths. Above all, we stress that it is pointless to attempt to reach detailed conclusions about cross-bridge movement by modelling studies alone until all the main components of the unit cell have been characterised adequately.

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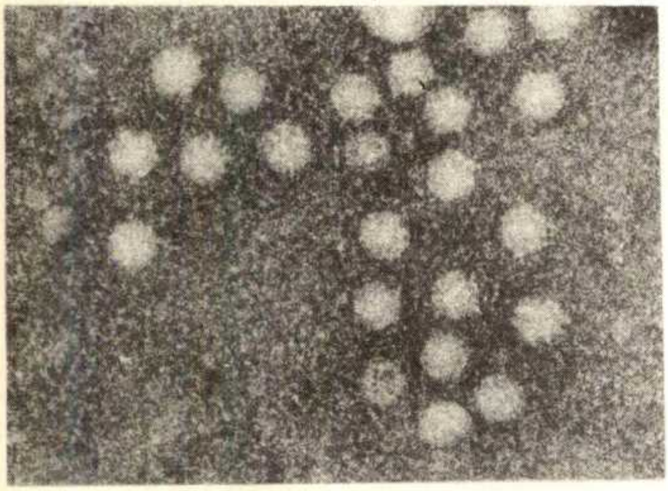
Morphology of the GB hepatitis agent

ATTEMPTS to isolate hepatitis A virus have been in progress for several decades. An important advance was the finding that the marmoset served as a permissive animal host for this virus[1], but it became obvious that more than one agent was active. The pedigree of one agent was established as MS-1 (hepatitis A) isolate, while the other, which was demonstrably different by neutralisation and cross-challenge experiments, was designated GB. The GB infectious serum was obtained on the third day of jaundice from a surgeon in Chicago who developed a mild form of acute hepatitis. This serum induced hepatitis in all four marmosets inoculated. A pool of infectious GB serum marmoset (passage 11) was prepared from the total bleeding of nine white-lipped marmosets (*Saguinus* sp.) 19–24 d after inoculation and during the early acute phase of hepatitis as indicated by an increase of serum transaminase levels. This pool, designated H 205 GB pass 11, has an infectivity of $10^{4.5}$ marmoset ID_{50}. We have now examined the GB agent electron microscopically, and found that it is smaller and more fragile than the MS-1 strain[2], and therefore also the identical CR326 isolate[3] of hepatitis A, which have the morphological appearances of recognised, small cubic viruses measuring about 27 nm in diameter.

Serum for examination was chosen from the period of maximum infectivity—$10^{4.9}$ ID_{50}—as established by further passage in marmosets. Because immune electron microscopy has proved particularly useful in the investigation of viral hepatitis[4], we examined the sera in the presence of convalescent serum from marmosets. Preinfection and normal marmoset sera were used as controls.

Fig. 1 Aggregate of small spherical particles 20–22 nm in diameter found in the highest infectivity serum of marmoset infected with the GB agent. In this particular specimen the infectious marmoset serum had been reacted with recovery serum. × 275,000.



A prolonged search of the infectious serum reacted with convalescent serum revealed a few relatively small aggregates (immune complexes) of particles (Fig. 1), which were spherical and resembled parvoviruses. But aggregates were also found in infectious serum that had been reacted with normal serum. This implied that the infectious serum itself contained immune complexes. Subsequent examination of the serum itself confirmed that this was correct. Figure 2 illustrates an immune complex in GB-positive marmoset serum, revealing another distinctive feature of this virus. Several of the particles showed the rim staining associated with empty capsids and some of the empty capsids were fragmented further to reveal crescent-shaped forms.

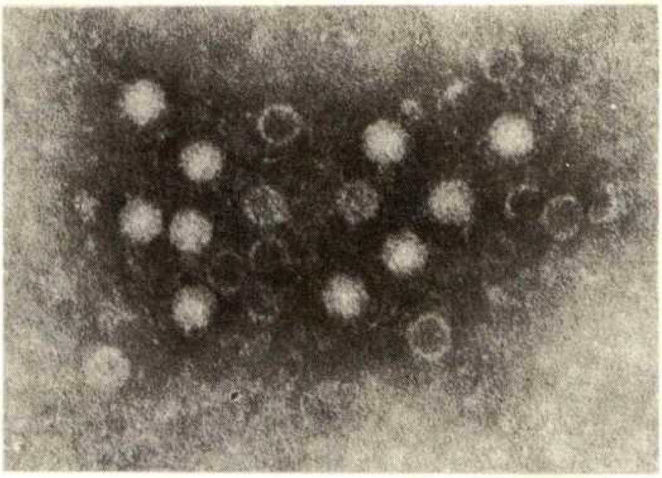


Fig. 2 Aggregate of 20–22-nm particles present in marmoset serum with no added antibody. This complex shows empty particles with ring staining and those even further degraded to crescentic forms. × 275,000.

These findings are interesting from two points of view: morphology and immunology. Having recently examined the MS-1 hepatitis A isolate in chimpanzee faeces[5], we have been able to establish that there is a considerable size difference between MS-1 and GB isolates. MS-1 has a peak size distribution between 28 and 30 nm, whereas the GB isolate has its peak size distribution between 20 and 22 nm. In addition, the GB agent seems to be more fragile, as the crescent-shaped forms seen in Fig. 2 were a recurrent feature, whereas they were not seen with MS-1. The morphological characteristics of GB are in accord with filtration data[6] which suggested that the virus was about 20 nm in diameter. The second point of interest concerns the fact that the virus was present in the serum in the form of immune complexes. This finding too was in good agreement with published data. Hilleman[7] demonstrated brief anticomplementary activity in early acute phase sera of hepatitis A illness and extrapolated that immune complexes might also form in the course of this infection.

The marmoset sera examined were near the limits of sensitivity of direct negative staining for electron microscopy. An infectivity titre of $10^{4.5}$ ID_{50} per ml might be considered too low for visualisation of virus. It may have been the aggregates that made the virus visible. Similar structures were not seen in normal marmoset sera.

There is considerable interest, particularly in the United States, in the possibility that not all cases of viral hepatitis can be classified as either A or B. This has led to the suggestion of a third group, which could consist of several conditions and which at present is termed either human viral hepatitis type C[8] or non-A:non-B hepatitis[9]. The GB agent that we describe here does not belong morphologically to either hepatitis A or B and it may well be a third agent causing viral hepatitis in man.

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Immunological cross reactivity of Mason–Pfizer monkey virus with type C RNA viruses endogenous to primates

THE Mason–Pfizer monkey virus (MPMV) was originally isolated from a spontaneous mammary carcinoma of a rhesus monkey[1,2]. Further isolates have been described from normal and malignant rhesus monkey tissues[3]. There have also been reports of viruses[4-7], nucleic acids[8] and antigens[9] related to MPMV in human tissues. Viruses of the MPMV group have a density in sucrose of 1.16 g cm^{-3} (refs 10 and 11) and possess a segmented 60–70S RNA viral genome[10-12] which contains polyadenylate sequences of about 200 nucleotides[13]. In addition, MPMV contains a reverse transcriptase [14,15] and structural polypeptides whose number and molecular weights resemble those of known type C RNA viruses[16,17]. In spite of these similarities with type C viruses, available evidence indicates that MPMV falls into a distinct group. This conclusion is based on morphological features that distinguish MPMV from type C viruses[18], as well as differences in the biochemical properties of their reverse transcriptases[15]. Further, MPMV lacks detectable immunological cross reactivity with the 30,000 molecular weight (p30) major structural polypeptides of mammalian type C viruses[5,16]. The antigenic determinants shared by p30s of known mammalian type C viruses have been used extensively in the identification of new virus isolates and in the analysis of immunological relationships between type C viruses[19,20]. Other type C virus structural components such as gp70 also have been shown to possess broadly reactive antigenic determinants[21,22]. We have now purified and characterised the gp70s of an endogenous virus of the baboon, an old world primate and of MPMV. Our results provide evidence that MPMV is immunologically related to endogenous type C viruses of primates.

Viruses, isopycnically banded in sucrose from tissue culture fluids, were provided by J. Gruber, Office of Resources and Logistics, Virus Cancer Program, NCI. Endogenous type C viruses of the *Papio cynocephalus* baboon[23] and of the cat, RD114[24], were used. Other type C viruses included Rauscher[25] and AKR-murine leukaemia virus (MuLV)[26], the Rickard strain of feline leukaemia virus (FeLV)[27], and infectious type C viruses of the woolly monkey[28] and gibbon ape[29]. MPMV was grown in human or rhesus monkey cells; type C viruses were propagated as indicated below. Antisera, prepared by immunisation of goats with Tween–ether–disrupted viruses, and swine anti-goat immunoglobulin G were given by R. Wilsnack, through the Office of Resources and Logistics, NCI.

The gp70s of baboon type C virus, grown in canine thymus cells, and of MPMV, propagated in human cells, were isolated as described before[22]. In addition, the major structural polypeptides of molecular weight 30,000 (p30) and 26,000 (p26) of the baboon type C virus and MPMV, respectively, were isolated from the same virus preparations according to published methods[20]. Each purified antigen was ^{125}I-labelled by the chloramine T method of Greenwood *et al.*[30] and shown by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate[31] to be at least 90% radiochemically pure.

Antisera prepared against various mammalian type C viruses and MPMV were first compared for their abilities to bind the gp70 and p30 structural polypeptides of the baboon virus and MPMV. As Table 1 shows, ^{125}I-labelled gp70 of the baboon virus was

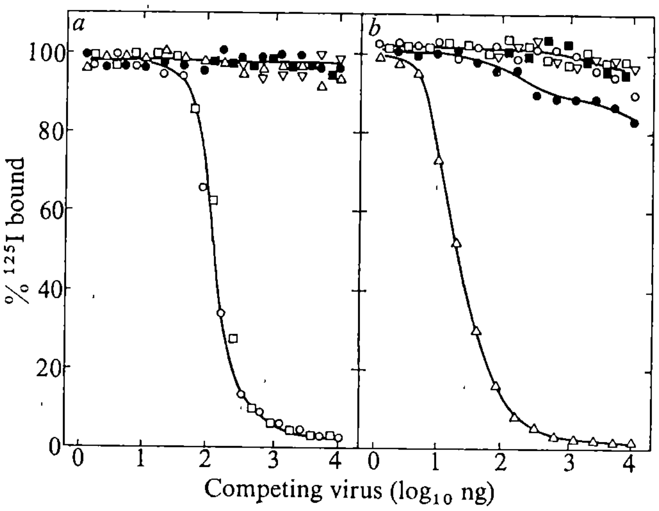

Fig. 1 Comparison of the immunological reactivities of MPMV and representative type C virus isolates of different mammalian species in homologous immunoassays for the 70,000 molecular weight glycoproteins of MPMV and of the endogenous *Papio cynocephalus* baboon type C virus. Unlabelled detergent-disrupted[20] viral antigens were tested at serial twofold dilutions for their ability to compete with ^{125}I-labelled viral gp70 for binding limiting amounts of antiserum. Reaction mixtures contained 0.01 M Tris–HCl, *p*H 7.8, 0.1 M NaCl, 0.001 M EDTA, 0.1% Triton X-100 and 1% bovine serum albumin in a volume of 0.15 ml. After incubation of antiserum and unlabelled competing viral antigen at 37 °C for 1 H, the appropriate ^{125}I-labelled viral gp70 was added in 0.05 ml and incubation continued for 3 h at 37 °C and a further 18 h at 4 °C. Undiluated swine anti-goat immunoglobulin G (0.025 ml) was then added to each tube and reaction mixtures were incubated for 3 h at 4 °C, centrifuged at 2,500 r.p.m. for 15 min, and the resulting precipitate was analysed for radioactivity. The viruses tested included MPMV propagated in either human (○), or rhesus monkey cells (□), and type C viruses including baboon (△), RD114 (●), gibbon ape (▲) and woolly monkey viruses (■), all grown in human cells and Rauscher MuLV grown in mouse cells (▽). Viral protein concentrations were determined by the method of Lowry *et al.*[36] *a*, Homologous MPMV immunoassay in which limiting antiserum against MPMV was used with ^{125}I-labelled MPMV gp70, *b*, homologous baboon type C virus gp70 immunoassay in which limiting antiserum against baboon virus was used with ^{125}I-labelled baboon viral gp70.

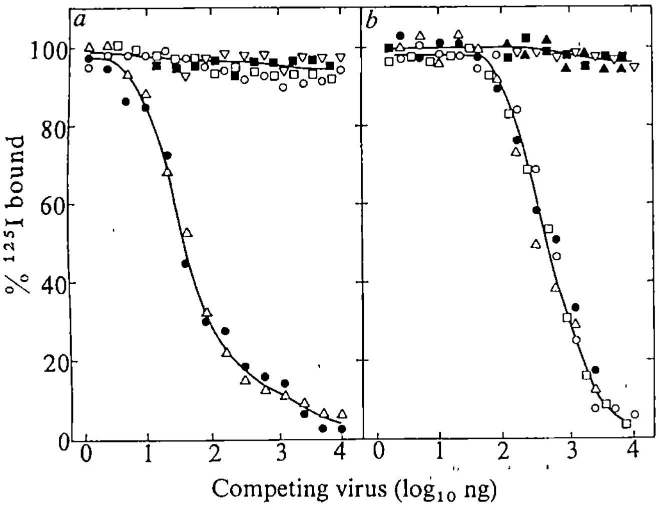

Fig. 2 Interspecies competition immunoassays for the 70,000 molecular weight glycoproteins of MPMV and the baboon type C virus The viruses tested and symbols used for each are indicated in the legend in Fig 1. Immunoassays were performed as described in Fig 1, and included: *a*, heterologous immunoassay in which limiting antiserum to RD114 was used with ^{125}I-labelled baboon virus gp70, and *b*, heterologous immunoassay in which limiting antiserum to MPMV was used with ^{125}I-labelled baboon type C virus gp70.

precipitated at titres of 1:10,000 and 1:150,000, respectively, by antisera to the endogenous baboon virus and to a closely related endogenous virus of the cat, RD114. The maximum extent of precipitation was about 90% with each antiserum These results indicated preservation, during purification, of antigenic determinants of the baboon viral gp70. The precipitation titres of the same antisera against baboon viral p30 were 10- – 20-fold greater, consistent with previous observations that antisera to type C viruses prepared in heterologous hosts exhibit higher titres against p30 than gp70 (ref. 21). Antisera to several other mammalian type C viruses also precipitated baboon viral p30 at titres ranging from 1:2,900 with anti-AKR–MuLV to 1:18,000 with antiserum to FeLV None of these antisera, however, showed detectable reactivity against baboon viral gp70 (titres less than 1:10)

As Table 1 shows, two separately prepared antisera to MPMV each demonstrated a high level of reactivity against ^{125}I-labelled MPMV gp70. The maximum extent of precipitation was around 70%, and titres for 10% binding were 1:450,000 and 1:280,000, respectively The immunoprecipitation titres of these same antisera against the major structural polypeptide of MPMV were even higher It is interesting (Table 1) that both antisera also demonstrated significant binding of baboon viral gp70. The precipitation titres were 1:640 and 1:400, with maximum precipitation of 69% and 53% of the labelled baboon viral gp70 probe. The anti-MPMV sera also precipitated ^{125}I-labelled RD114gp 70 at titres of 1:1,000 and 1:800, but showed no detectable reactivity against the gp70s of FeLV, Rauscher–MuLV or woolly monkey virus (data not shown). These results suggested that MPMV shares antigenic determinants with gp70s of viruses of the baboon-RD114 virus group but not with gp70s of other mammalian type C viruses. Findings that the same MPMV antisera failed to precipitate p30s of either the baboon virus (Table 1) or Rd114 (data not shown) are consistent with previous reports[5,16], indicating lack of relatedness of the major structural polypeptide of MPMV with analogous polypeptides of mammalian type C viruses.

To characterise the gp70 antigenic determinants cross reactive of competition immunoassays were developed. As Fig. 1*a* shows, in an immunoassay utilising anti-MPMV serum to precipitate ^{125}I-labelled MPMV gp70, MPMV, propagated in either human or rhesus monkey cells, competed with high efficiency. In contrast, several type C viruses tested, including baboon and RD114, showed no detectable reactivity. In an immunoassay utilising antiserum to the baboon virus to precipitate ^{125}I-labelled baboon viral gp70 (Fig 1*b*), only the homologous virus competed efficiently RD114 competed 10–15% of the ^{125}I-labelled gp70 at the highest virus concentration tested, while none of the other mammalian type C viruses or MPMV showed any reactivity Thus, MPMV and baboon viral gp70s could be distinguished readily in these two assays

Immunoassays designed to detect more broadly reactive antigenic determinants of the baboon viral gp70 utilised antisera against RD114 or MPMV to precipitate ^{125}I-baboon viral gp70. Reciprocal studies with ^{125}I-MPMV gp70 were not possible because available antisera to baboon-RD114 viruses failed to precipitate this polypeptide As Fig. 2*a* shows, baboon and RD114 viruses competed with similar high efficiency in the heterologous anti-RD114: ^{125}I-baboon viral gp70 immunoassay, whereas MPMV and the other type C viruses tested were all unreactive.In the anti-MPMV: ^{125}I-baboon viral gp70 assay (Fig 2*b*), MPMV, baboon and RD114 viruses each competed with similar efficiency, although none of the other type C viruses, including several grown in human or rhesus monkey cells, showed any detectable reactivity Purified MPMV and baboon viral gp70s were found to possess reactivities identical to those of their respective parental viruses in each of the competition immunoassays used in the studies reported here (data not shown) These findings indicate that the shared antigenic determinants of MPMV and the baboon type C virus were associated with 70,000 molecular weight polypeptides of each virus

Two lines of evidence exclude the possibility that the immunological cross reactivity of MPMV with baboon and RD114

Table 1 Comparison of abilities of antisera to bind ^{125}I-labelled MPMV and baboon virus polypeptides

Antisera to	Cells propagated in	Antisera titres for binding ^{125}I-labelled polypeptide of*							
		baboon virus gp70		baboon virus p30		MPMV gp70		MPMV p30	
		titre	maximum precipitation (%)	titre	maximum precipitation (%)	titre	maximum precipitation (%)	titre	maximum precipitation (%)
Baboon virus	Human	10,000	90	200,000	88	<10	<5	<10	<5
RD114	Human	150,000	89	1,200,000	90	<10	<5	<10	<5
Gibbon ape virus	Human	<10	<5	5,000	70	<10	<5	<10	<5
Woolly monkey virus	Human	<10	<5	12,000	85	<10	<5	<10	<5
FeLV	Cat	<10	<5	18,000	67	<10	<5	<10	<5
Rauscher MuLV	Mouse	<10	<5	4,100	75	<10	<5	<10	<5
AKR-MuLV	Mouse	<10	<5	2,900	80	<10	<5	<10	<5
MPMV	Rhesus monkey	640	69	<10	<5	450,000	70	1,600,000	60
MPMV	Human	400	53	<10	<5	280,000	68	1,700,000	61

* ^{125}I-labelled viral polypeptides (10,000 c.p.m.) were incubated with antisera at serial twofold dilutions in reaction mixtures containing 0.01 M Tris-HCl, *p*H 7.8, 0.1 M NaCl 0.001 M EDTA, 0.1% Triton X-100 and 1% bovine serum albumin, in 0.2 ml After 3 h incubation at 37 °C and a further 18 h at 4 °C reaction mixtures were diluted to 1.0 ml, mixed with 0.1 ml pig anti-goat immunoglobulin G, and incubated for 3 h at 4 °C The immunoprecipitates were centrifuged at 2,500 r.p.m. for 15 min and analysed for radioactivity Antiserum titre is expressed as reciprocal of the highest serum dilution able to bind 10% of the appropriate ^{125}I-labelled viral polypeptide Maximum precipitation values represent the percentage of ^{125}I-labelled antigen bound at the highest antiserum concentration tested

viruses was due to contamination of MPMV stocks with a known type C virus. First, the patterns of reactivity of each virus in the various competition immunoassays for gp70 used in these studies distinguished the type C viral cross-reactive determinants of MPMV from those of baboon or RD114 viruses or any of several other type C viruses tested Furthermore, the MPMV preparations used here did not cross react in immunoassays[5,16] which sensitively detect the major structural polypeptides of all known mammalian type C viruses (data not shown).

The results thus indicate that there must either be antigenic relatedness of virus-coded polypeptides of MPMV and type C viruses of the baboon-RD114 group or that these viruses incorporate some cross-reactive cellular protein The preservation of immunological characteristics of a virion polypeptide after virus growth in cells of very different species is generally considered strong evidence that the polypeptide is virus coded. This criterion could be met readily for baboon and RD114 viruses. These viruses have a wide host range, and the antigenic specificities of their gp70s were stable when they were grown in cells of diverse hosts Since MPMV is only infectious for cells of primate origin, this criterion cannot as adequately be satisfied for MPMV gp70. But the absence of the cross-reactive antigen in other viruses grown in human or rhesus monkey cells excludes the nonspecific incorporation of this antigen by MPMV Moreover, our results indicate that purified MPMV gp70 displayed the same antigenic specificities as the intact virus Thus, if the type C viral cross-reactive antigen associated with MPMV is cellular rather than MPMV coded, it must specifically be associated with MPMV and possess physical properties very similar to those of MPMV gp70.

The more likely alternative is that MPMV gp70 has both MPMV-specific antigenic determinants as well as others that are shared with type C viruses of the baboon-RD114 group. As previous findings have indicated, the reverse transcriptase[15] and major structural antigen[5,16] of MPMV lack detectable cross-reactivity with analogous components of known mammalian type C viruses. The rhesus monkey, a natural host of MPMV, contains genetic information for an endogenous type C virus related to the baboon type C virus[32] and expresses antigens of this virus in its normal tissues[33] Moreover, experimental evidence has established that recombination can occur between infectious type C viruses and endogenous type C viruses of the cells in which they are propagated[34,35]. Thus it can be speculated that MPMV may have initially acquired its gp70 by a mechanism involving recombination with an endogenous primate type C virus

Like MPMV, several reverse transcriptase-containing viruses, including primate syncytial viruses, visna virus and mouse mammary tumour virus, have morphological characteristics that differ from those of type C viruses. So far studies have failed to establish an immunological relationship of any of these viruses with mammalian type C viruses in tests of either their reverse transcriptases or major structural polypeptides. The application of radioimmunological techniques for detection of other viral components should help to determine whether any of these viruses like MPMV, is immunologically, and thus possibly evolutionarily, related to type C RNA viruses.

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Newcastle disease virus mRNA lacks 2′-*O*-methylated nucleotides

MESSENGER RNA molecules from various sources contain methylated nucleotides at the 5′ terminus of the RNA molecules[1–9]. The structure at the 5′ terminus of most cellular and viral mRNAs consists of a terminal base-methylated nucleotide linked to a ribose-methylated nucleotide through a 5′ to 5′ pyrophosphate bond, such as $m^7G(5')ppp(5')AmpNp$ or $m^7(5')ppp(5')GmpNp$. Initiation and subsequent *in vitro* translation of mRNA are dependent on the presence of m^7G in a blocked 5′ structure[10,11]. Thus, methylation (m^7G) seems to serve an important function in the translation (and/or control) of viral and cellular mRNA. The role of 2′-*O*-methylation has not been identified The widespread presence of 2′-*O*-methylation in mRNA from diverse systems suggested that they might be a common feature of all eukaryotic mRNAs[4–9]. We report here that the 5′-terminal structure of Newcastle disease virus (NDV) mRNA synthesised *in vitro* is $m^7G(5')ppp(5')GpPyp$. The structure isolated from NDV mRNA differs from those reported for all other mRNAs synthesised *in vitro*[5–8] in that the RNA lacks 2′-*O*-methylated nucleotides. During the preparation of our report we learned that the 40S RNA genome of Sindbis virus[14] and the 26S RNA synthesised in Sindbis virus-infected cells[15] have the structure $m^7G(5')pppNp$. In addition tobacco mosaic virus has $m^7G(5')ppp(5')Gp$ at the 5′ end of the viral genome[12,13].

Newcastle disease virus (NDV), and avian paramyxovirus contain a virion-associated RNA polymerase[16] that transcribes the 50S RNA genome *in vitro* into 18S molecules. These molecules are complementary in base sequence to the genome and contain polyadenylic acid sequences[17]. We[18,19] have demonstrated the presence of a methyltransferase activity in purified NDV which catalyses the incorporation of the methyl group from ^3H-S-adenosyl-L-methyl methionine (SAM) into the messenger RNA synthesised *in vitro* by the virion-associated transcriptase. The procedures for growth and purification of NDV, the reaction mixture for *in vitro* RNA synthesis in the presence of SAM, the methods for isolation and purification of the *in vitro* RNA product, and the DEAE-cellulose chromatography for analysis of oligonucleotides have been described before[18].

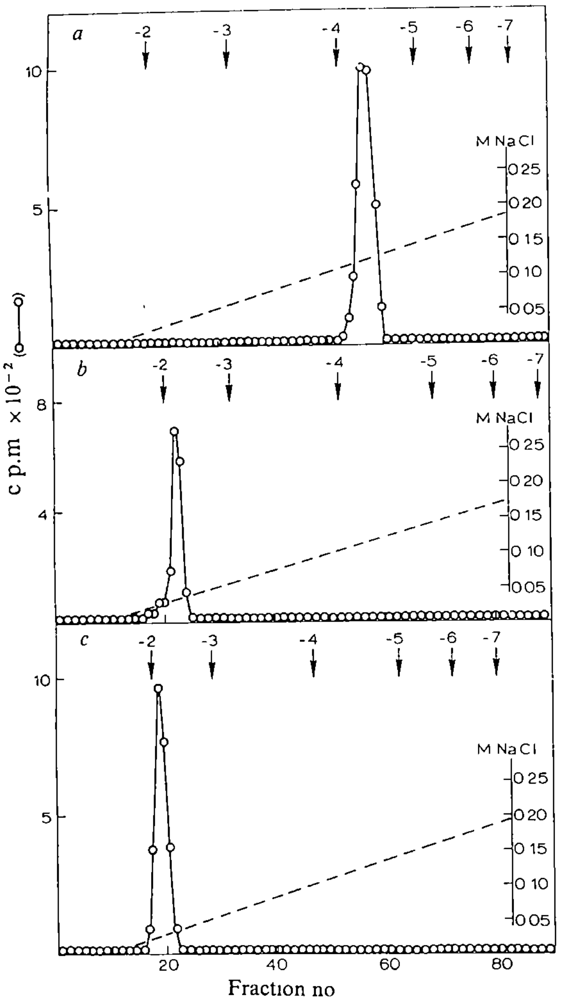

Fig. 1 DEAE-cellulose chromatography of ^{3}H-methyl-labelled RNA products after enzymatic digestions. *a*, Purified ^{3}H-methyl-labelled RNA was denatured in a boiling water bath for 3 min and a 0.5-ml sample in 10 mM Na acetate, *p*H 4.5, was incubated with 4 U of RNase T$_2$ (Calbiochem) at 37 °C for 5 h. The digest was chromatographed on a 0.6×17-cm column of DEAE-cellulose with 3 mg of RNase A-digested rat liver ribosomal RNA as an oligonucleotide marker and a portion of each fraction was analysed for radioactivity as described before[18]. The salt gradient is indicated by the dotted line. The elution positions of marker oligonucleotides are indicated by arrows and expressed as their net negative charges. The fractions containing radioactivity were desalted by dialysis against water, lyophilised, resuspended in buffer, and a portion of the RNase T$_2$-resistant oligonucleotide was digested with either alkaline phosphatase or penicillium nuclease before rechromatography. *b*, Alkaline phosphatase digest of (*a*). A sample (0.5 ml) of the oligonucleotides obtained from the radioactive region shown in (*a*) was incubated in 20 mM Tris-HCl, *p*H 8.0, with 2.5 mg (125 U) of bacterial alkaline phosphatase (Worthington) for 1 h at 37 °C. The sample was then chromatographed on DEAE-cellulose as in (*a*). *c*, Penicillium nuclease digest of (*a*). A sample (0.5 ml) of the oligonucleotides obtained from the radioactive region shown in (*a*) was incubated in 10 mM Na acetate, *p*H 6.0, with 100 µg of penicillium nuclease (Yamasa Shoyu Co.) for 1 h at 37 °C. The sample was then chromatographed on DEAE-cellulose as in (*a*).

To determine the specific site of methylation, ^{3}H-methyl-labelled RNA was synthesised in the presence of SAM and then digested with RNase T$_2$ (which degrades RNA to nucleoside-3′-monophosphates in the absence of 2′-*O*-methyl groups), and the resulting products were analysed on a DEAE-cellulose column. A single peak (Fig. 1*a*) of ^{3}H radioactivity eluted between the −4 and −5 charged markers identical to the position previously reported[18] for the ^{3}H-methyl-labelled RNA after KOH hydrolysis. Structures which should elute in the position described include pNmpNp, mNpppNp or NppNmpNp. The labelled material was desalted and a portion was treated with bacterial alkaline phosphatase (which removes terminal monoesterified phosphate groups) and reanalysed on DEAE-cellulose. A single peak of radioactivity now eluted between the −2 and

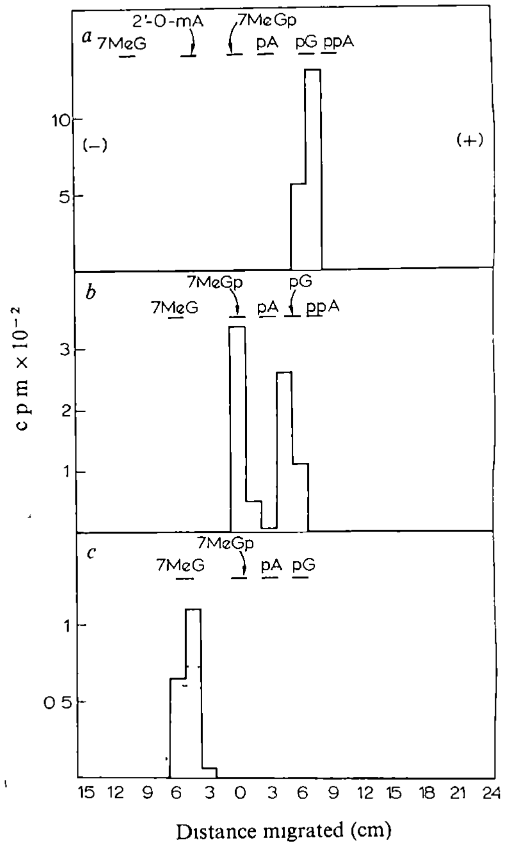

Fig. 2 Paper electrophoresis of the terminal oligonucleotide. *a*, Purified ^{3}H-methyl-labelled RNA was digested with penicillium nuclease as described in Fig. 1*c* and then examined by high voltage paper electrophoresis using a procedure similar to that of Abraham *et al*[8]. Unlabelled markers (20 µg in 2 µl) and labelled samples (2–10 µl) were spotted on Whatman 3 MM paper (33×39 cm) and separated by electrophoresis in pyridine: acetic acid: water (1:10:89), *p*H 3.5, at 1,200 V for 2 h, using a Brinkmann MINI 68 high voltage cold plate electrophoresis apparatus. The paper was then dried and the markers located under ultraviolet light. The paper was cut into pieces (1.5 cm×1.5 cm) and the radioactivity in each piece was counted in 10 ml of toluene containing PPO and POPOP. *b*, The ^{3}H-labelled material migrating as pG (*a*) was recovered from the paper as described below, digested with nucleotide pyrophosphatase as follows and then subjected to high voltage paper electrophoresis. Paper squares which contained radioactivity were removed from the counting vials, washed twice in toluene to remove fluors and dried. One side of the square was cut to form a point and water was allowed to ascend up to the point. The square was wrapped in aluminium foil (point sticking out) and suspended point down in a test tube so that the square was 1 cm from the bottom of the tube. Labelled samples were then recovered by centrifuging in an International PR-2 centrifuge (1 min, 1,000 r.p.m.). The labelled sample was digested with 0.025 U of nucleotide pyrophosphatase in 0.5 ml 20 mM Tris-HCl, *p*H 7.5, 1 mM MgCl$_2$ for 15 min at 37 °C. The sample was lyophilised, resuspended in 10 µl of water and spotted on Whatman 3 MM paper. Electrophoresis and assay were performed as in (*a*). *c*, The labelled materials migrating as m^{7}Gp and pG (*b*) were recovered from the paper, digested with alkaline phosphatase, and then analysed by high voltage paper electrophoresis. The radioactivity was recovered from the paper squares as described in (*b*), and alkaline phosphatase digestion was performed as described in Fig. 1*b*. The samples were lyophilised, resuspended in 10 µl of water and spotted on Whatman 3 MM paper. Electrophoresis and assay of the materials which had migrated as m^{7}Gp(–) and pG(- - -) were performed as in (*a*).

Table 1 Periodate oxidation and removal of the blocking base by aniline

RNA Sample	C P.M Treated	C.P M (%) Recovered in ethanol*	C.P.M (%) Recovered in precipitated RNA
^{14}C-UMP-labelled 18+28S rRNA	9.700	824 (8.6)	8,795 (91.4)
^{3}H-methyl-labelled product RNA	4,400	4,029 (92.1)	347 (7.9)

*Values in this column represent the total c.p m recovered from four ethanol precipitations.

—3 charged markers (Fig. 1*b*), indicating the removal of a single phosphate group This result eliminates structures such as pNmpNp because two phosphate groups would have been removed by alkaline phosphatase To distinguish between structures such as mNpppNp and NppNmpNp, a portion of the methyl-labelled oligonucleotide eluting in Fig. 1*a* was treated with penicillium nuclease (which degrades RNA to nucleoside-5′-monophosphates, regardless of the presence of any 2′-*O*-methyl groups, and also contains a 3′-phosphatase activity), and analysed by DEAE–cellulose chromatography. The elution pattern (Fig. 1*c*) did not differ from the pattern obtained after RNase T_2 digestion and alkaline phosphatase treatment (Fig. 1*b*), suggesting the absence of any 2′-*O*-methyl groups.

The ^{3}H-methyl-labelled structure at the 5′ terminus of NDV mRNA was analysed further by high voltage paper electrophoresis. After treatment with penicillium nuclease, the resulting material migrated on paper near the pG marker (Fig. 2*a*) This material was eluted from paper, treated with nucleotide pyrophosphatase (which cleaves pyrophosphate linkages), and reanalysed by paper electrophoresis Treatment with nucleotide pyrophosphatase released two equally labelled products (Fig. 2*b*), one of which comigrated with the pG marker These two products most likely represent m^{7}Gp and m^{7}Gpp, respectively Both products were recovered, treated with alkaline phosphatase, and reanalysed by paper electrophoresis. Treatment with alkaline phosphatase removed the remaining phosphate groups, and ^{3}H-methyl radioactivity of both products migrated with authentic m^{7}G (Fig. 2*c*). These results show conclusively that the ^{3}H-methyl label resides solely in m^{7}G. The fact that two products were obtained after nucleotide pyrophosphatase digestion (Fig. 2*b*) proves that the 5′ terminus contains three phosphates. Cleavage of a diphosphate would have given a single product.

Thus the methylation of NDV complementary RNA exists as a m^{7}G and can be depicted as m^{7}GpppNp, Npppm^{7}Gp or m^{7}Gpppm^{7}Gp. If a 5′–5′ linkage exists between the two nucleosides, the blocking nucleoside (m^{7}G or N) will contain free 2′- and 3′-hydroxyl groups and will be subject to periodate oxidation and removal by treatment with aniline[8]. When ^{3}H-methyl-labelled RNA was oxidised with potassium periodate, followed by treatment with aniline, less than 8% of the methyl radioactive label was recovered with ethanol-precipitated RNA (Table 1), indicating that the m^{7}G resides as a blocking base. As a control for experimental manipulations, parallel treatment of ^{14}C-UMP-labelled 18 and 28S ribosomal RNA resulted in more than 91% recovery of the radioactive label in precipitated RNA (Table 1) These results indicate that the blocking base is m^{7}G. The fact that almost all the radioactivity was removed eliminates m^{7}Gpppm^{7}G, because only 50% of the radioactivity would have been removed from the latter structure. Thus the 5′-terminal structure must be m^{7}G(5′)pppNp. Since more than 90% of the methyl label in NDV mRNA is released as m^{7}G by periodate and aniline treatment, and since the data in Fig 1 suggest the absence of any 2′-*O*-methyl groups, the level of any additional methylation, such as internal base methylations (N^6-methyladenosine[2–4] or 5-methyl cytosine[15]), cannot exceed 8% (the amount of methyl label product which was not released by periodate and aniline treatment, Table 1).

To identify the unknown nucleotide in the methylated 5′-terminal structure, ^{3}H-methyl-labelled RNA was treated with either RNase A or RNase T_1 and analysed on DEAE–cellulose columns Treatment with RNase T_1 alone (which cleaves on the 3′ side of guanylic acid residues) resulted in a methylated, RNase-resistant structure which eluted between the —4 and —5 negatively charged markers (Fig. 3*a*) Since the elution position is identical to that obtained by base hydrolysis[18] and ribonuclease T_2 treatment (Fig. 1*a*), the second base in the capped structure must be G. RNA treated in a similar manner with RNase A (specific for the 3′ side of pyrimidine nucleoside linkages) eluted between the —5 and —6 negatively charged markers (Fig. 3*b*). Thus the 5′ terminus of NDV RNA seems to be m^{7}G(5′)ppp(5′)GpPyp.

Since NDV mRNA does not contain any 2′-*O*-methylated nucleotides, it should serve as a valuable control in experiments designed to elucidate the role of 2′-*O*-methylated nucleotides in other eukaryotic mRNAs

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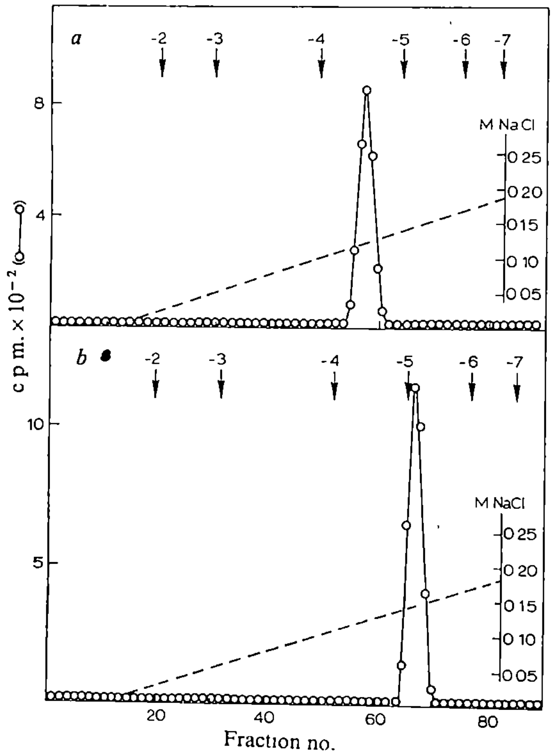

Fig. 3 DEAE–cellulose chromatography of ^{3}H-methyl-labelled RNA after RNase digestion. Purified ^{3}H-methyl-labelled RNA was digested with RNase T_1 (*a*) or RNase A (*b*) and chromatographed on DEAE–cellulose as described in Fig. 1. Labelled samples were resuspended in 0 5 ml of 5 mM Tris-HCl, *p*H 7.5, 1 mM EDTA, 0 3 M NaCl and incubated with 2 μg (678 U ml^{-1}) RNase T_1, or with RNase A (50 μg ml^{-1}) at 37 °C for 30 min. The salt gradient is indicated by the dotted line The elution positions of marker oligonucleotides are indicated by arrows and expressed as their net negative charges.

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DNA endoreduplication and polyteny understood as evolutionary strategies

DNA endoreduplication and related phenomena (such as endomitosis, polyteny, nuclear restitution and somatic polyploidy in general) are widespread over the animal and plant kingdoms, although they occur most frequently among insects and angiosperms[1-3]. The systematic restriction to certain phyla and species has been interpreted in terms of high genetic control of such events[3], whereas the characteristic developmental pattern of various degrees of endopolyploidy has been considered as an expression of their functional role in differentiation and synthesising capacity of the cells[2,4,5]. Recently, however, any role of endoreduplication in cell differentiation has been questioned because of the existence of species apparently lacking endopolyploidy[6]. All previous discussions on endoreduplication, endopolyploidy and polyteny have, however, ignored the basic DNA contents of the species studied. We here report a relationship between the basic nuclear DNA content and the occurrence and degree of endopolyploidy. This strongly suggests that DNA endoreduplication can be regarded as an evolutionary alternative to the high nuclear DNA content that has been achieved in other species mainly by 'saltatory replications'.

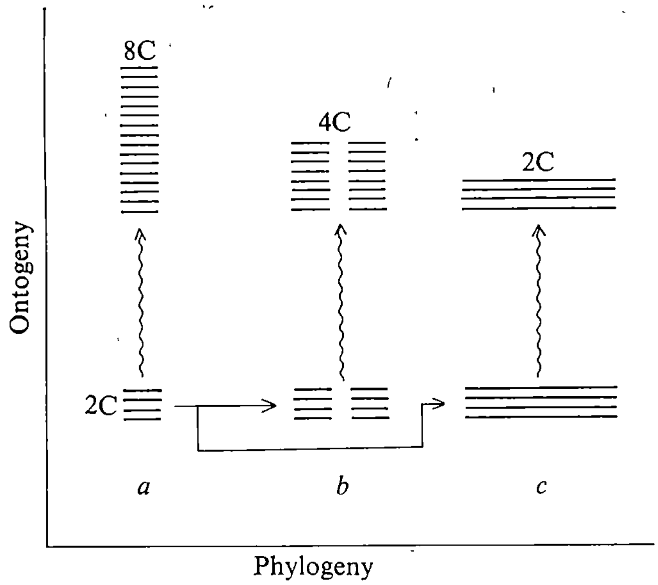

Fig. 1 Diagram to illustrate the relationship between phylogenetic increase in basic (2C) nuclear DNA and ontogenetic increase by somatic polyploidy The scheme assumes a diploid species with 4 chromosomes and low DNA content (*a*), a generative-tetraploid species with twice as many chromosomes and DNA (*b*), and a diploid species with 4 enlarged chromosomes with 4 times the DNA content of species a (*c*) During differentiation the most active cells need a certain DNA mass which is essentially similar in all species of comparable complexity To achieve this amount of DNA, 4 endocycles must occur in species *a*, two in species *b*, but none in species *c*. The scheme also suggests that phylogenetic DNA increase occurs tandemly, while ontogenetic increase occurs laterally. Polyteny is, therefore, the automatic consequence of DNA endoreduplication, if the sister chromosomes are not secondarily separated from each other, for example, by endomitotic chromosome coiling Note that species *b* may, in addition to generative polyploidy, develop 8C nuclei during ontogeny. Moreover, the trend to endopolyploidy may also increase, if the 2C DNA content is reduced during phylogeny (for example in the evolution of species)

As well as reviewing the huge literature on endopolyploidy, we have examined several species for the first time, using a Leitz MPV-2 scanning photometer interfaced to a PDP-8 computer (Digital Equipment). The following relationships could be detected. The lower the basic (2C) nuclear DNA content of a given species, the higher is the probability that endopolyploid or polytenic nuclei are developed in certain cells (Table 1). This negative relationship between 2C DNA content and maximum ploidy is surprisingly clear in relation to the rather small sample of animals and plants for which both data are available. Moreover, the same relationship is found among higher taxonomic groups (Table 2): Ciliata and Diptera, which have only low basic nuclear DNA contents, show a high tendency for endopolyploidisation and polytenisation; mammals and dicotyledonous plants, with intermediate amounts of DNA also develop, on an average, more intermediate levels of endopolyploidy; and Amphibia and monocots that possess the highest nuclear DNA contents, fail to show—at least higher—polyploidy levels.

Regarding the tissue-specific pattern of endopolyploidy and polyteny, the cells which display the highest levels are those which have been assumed to be the most active and specialised, such as salivary glands of insect larvae, the trophoblasts of mammalian embryos, the trophocytes of insect ovarioles, the suspensors of angiospermal embryos, and so on[8]. In this context it is worth mentioning that cases of differential DNA

Table 1 Nuclear DNA contents and maximum endopolyploidy levels in various plants and animals

Species	DNA (10^{-12} g per 2C nucleus)	Endopolyploidy (maximum C level)
Capsella bursa-pastoris	1.7	1,024*
Cucurbita pepo	2.6	384
Phaseolus coccineus	2.7	8,192
Phaseolus vulgaris	2.7*	2,048
Tropaeolum majus	2.7*	2,048*
Helianthus annuus	6.1–9.9*	16*
Hordeum vulgare	13.4	256
Lathyrus ssp.	9–20	64
Vicia faba	23.9–28.7	16
Allium cepa	35.5	32
Tradescantia virginiana	54.0	8
Pinus sylvestris	93.8	—
Picea alba	100.0	—
Pinus resinosa	138.0	—
Drosophila melanogaster	0.36	2,048
Chironomus tentans	0.5	32,768
Bombyx mori	1.0	524,288
Aplysia californica	2.0	200,000
Rattus rattus	6.1	4,096
Gryllus domesticus	12.0	16*
Triturus alpestris	58.0	—*
Salamander salamander	64.0	—*
Ambystoma mexicanum	76.0	—*

*DNA contents not taken from the literature but calculated from the data obtained by scanning densitometry of Feulgen-stained nuclei, using *Allium cepa* as a standard (2C = 35.5 pg (ref. 20)) to convert the relative values into pg. The other data have been taken from various reviews[8,18,21,22]. Negative results on endopolyploidy do not exclude the possibility that some specialised cells may, nevertheless, develop polyploid nuclei, which have not yet been detected. With a few exceptions, however, the data strongly indicate a negative relationship between basic (2C) nuclear DNA content and the maximum endopolyploidy reached within a species.

Table 2 Range of nuclear DNA contents and endopolyploidy levels in various vegetal and animal taxa (arranged in systematic order)

Taxon	Range of 2C DNA content (pg)[21-24]	Range of endopolyploidy[8]	Organ with the highest level[8]
Bryophyta	1.5–9.0	4–16	Paraphyses
Pteridophyta	30.0–300.0	—	—
Gymnosperms	8.0–200.0	—	—
Angiosperms	0.6–200.0	4–24,000	Suspensor, endosperm
Mollusca	1.0–10.0	4–200,000	Giant neurones
Insecta	0.3–15.0	4–500,000	Salivary glands, Malpighian tubules
Pisces	0.9–284.0	—	—
Amphibia	6.3–168.0	—	—
Mammalia	5.0–7.0	4–4,096	Trophoblast

The table shows that taxa with high average nuclear DNA contents, unlike those with rather low DNA amounts, do not show endopolyploidisation. The degrees of polyploidy reached in a species are, with a few exceptions, negativejy correlated to its basic nuclear DNA content (Table 1). This explains the high variation of ploidy levels seen in some taxa.

replication can be regarded as an economical and/or necessary variation of endoreduplication: DNA underreplication occurs in cells which no longer need the centromeric heterochromatin, probably because they will never divide again[8,9]. DNA amplification has been envisaged as a local, transitory polytenisation in germ line cells that must not undergo endopolyploidisation[10].

The following conclusions can be drawn from these findings. The differentiation and specialised function of certain cells of eukaryotic organisms evidently need a defined mass of DNA (and probably not only of protein-coding and ribosomal DNA) to maintain their regulatory and functional state. The necessary DNA content has been achieved in many species through two different ways: one is characterised by tandem duplications of DNA sequences (for example, by saltatory replications), leading to the increase of predominantly non-informative, reptitive DNA sequence families[7,11,12]. The other way is by generative (or germ-line) polyploidy[13], which yields, in addition, the advantage of possible gene dose effects in the 'laterally' multiplicated genome[14-17], as well as the possibility to overcome the general limit of DNA increase. A third way may be seen in the tandem arrangement of polyploid chromosome sets, leading to larger chromosomes in a stable number (cryptopolyploidy[18] which, however, is still a rather speculative idea).

When the phylogeny of an order, family or genus has not been accompanied by an increase in the basic nuclear DNA, however, the necessary DNA content is achieved during ontogeny, by various species- and tissue-specific degrees of somatic polyploidy. With regard to differentiated cells, this may result in a similar effect (Fig. 1). Thus, DNA endoreduplication, polyteny, and somatic polyploidy in general, evidently represent an evolutionary strategy which substitutes for a lack of phylogenetic increase in nuclear DNA. In spite of the evidence given for this interpretation in Tables 1 and 2 it should be generalised only with caution: it is a general feature of evolution that different strategies may be involved in the creation of even closely related species[19].

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Internal inversion used to study regulation of the *int* gene in bacteriophage λ

Two events are essential for successful lysogenisation by bacteriophage λ: establishment of repression and integration of the viral genome into the bacterial chromosome. Integration is catalysed by the phage Int function[1]. The *int* gene is situated at the distal end of the early leftward N

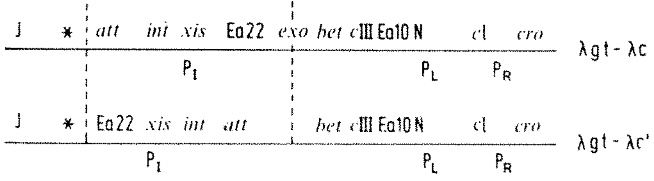
A
(a)
(b)
J b att int xis Ea22 exo bet cIII Ea10 N cI cro y cII O
P_I
O_L P_L P_R O_R P_{re}
B
R1-2
R1-3
J * att int xis Ea22 exo bet cIII Ea10 N cI cro
λgt-λc
P_I P_L P_R
J * Ea22 xis int att bet cIII Ea10 N cI cro
λgt-λc'
P_I P_L P_R

Fig. 1 Portion of the genetic map of bacteriophage λ showing transcription pattern and the structure of the reconstructed phages λgt–λC and λgt–λC′. *A*, After infection, transcription of the two early λ operons is initiated at P_L and P_R (arrows in (*a*)). In the establishment of lysogeny, transcription of the repressive gene originates at Pre and of the *int* gene probably at P_I. The synthesis of both transcripts (arrows in (*b*)) is activated by the positive regulators cII and cIII. *B*, *Eco*RI restriction sites and the genetic structure of the inversion has been determined before[5]. The stars denote the position of the b region of bacteriophage λ which was partly deleted during the construction of these phages. The genetic location of the genes responsible for the synthesis of the various protein bands was determined by the use of various known deletion[7] and amber mutations (in my work, in preparation), and is consistent with previous reports[8].

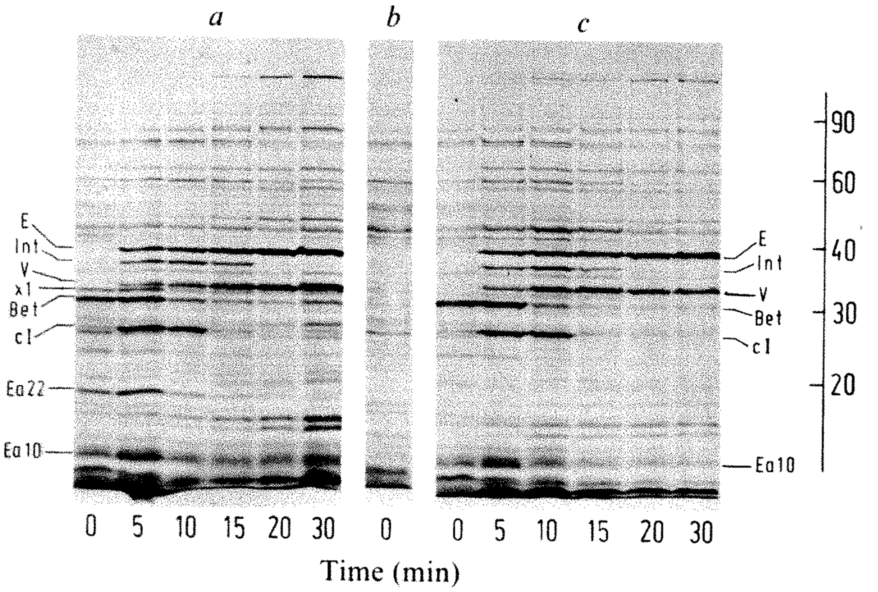

Fig. 2 Analysis of phage-directed protein synthesis. Cells were grown in HFCM medium[8], irradiated with 1.6×10^4 erg mm^{-2} and starved for 15 min at 37 °C before being infected at a multiplicity of 10. The infected cells were incubated in HFLA minimal medium[8] and pulsed with ^{14}C-mixed amino acids (5 μCi min^{-1}) for 5-min intervals starting at the time designated. Samples were subjected to electrophoresis on SDS–polyacrylamide slab gels[9,10] containing 20% polyacrylamide and 0.067% *bis*-acrylamide. After electrophoresis the gels were dried and exposed to X-ray film for 14 d for autoradiography. *a*, Infection with λgt-λC; *b*, uninfected control; *c*, infection with λgt-λC′. The molecular weight scale given on the right (in kdaltons) is based on the following standards: serum albumin, catalase, ovalbumin, γ globulin (two chains), trypsin, cytochrome *c*, and chymotrypsin (two chains). Identification of protein bands, except for the products of the late genes *E* and *V*, is based on the combined use of deletions and amber mutations. The discrepancy in molecular weight determination of Int, Ea22 and Ea10 with respect to published data is due to the difference in gel composition (my work, in preparation).

operon, whose transcription is initiated at the P_L promoter (Fig. 1). But in addition to P_L-promoted transcription, the *int* gene is specifically activated by the action of the *c*II and *c*III gene products, and we have proposed that the *c*II and *c*III proteins stimulate fast synthesis of Int by acting at the P_L promoter (my unpublished work with M. Katzir, M. Belfort and A. Oppenheim). In this respect the *int* gene behaves similarly to the *c*I repressor gene, whose fast expression is activated by the gene products of *c*II and *c*III[2,3].

Strains of λ have been constructed by rejoining *Eco*RI DNA fragments[4,5]. As cohesive ends generated by the *Eco*RI nuclease are symmetrical, in a reconstructed phage a DNA segment, not essential for phage growth, can be found either in the native or inverted form. λgt–λC′ is such a phage in which the *att–int–exo* region has been inverted[5] (Fig. 1). A gene within the inverted segment would be expected to express itself only if its promoter is also included within the inverted region. On the other hand, if the promoter of the gene in question is outside the inverted segment only "anti-sense" message will be transcribed and no functional protein will be produced. I have used this principle to define the location of the promoter for fast expression of the *int* gene.

Ultraviolet irradiated cells were infected by either λgt–λC or λgt–λC′, then pulse labelled with ^{14}C and subjected to electrophoresis as described in the legend to Fig. 2. Representative autoradiograms are shown in Fig. 2. Evidence for the identification of the various protein bands will be published elsewhere.

The results presented in Fig. 2 can be summarised as follows. First, the synthesis of the proteins coded by the genes *bet* and Ea10 (Fig. 1) was unaffected by the presence of the inverted segment (Fig. 2). Protein band Ea22 was present in the native phage (Fig. 2*a*) but absent from the inverted phage (Fig. 2*b*). This result was expected as the gene which is responsible for the synthesis of this protein band is present in the inverted segment. Second, the two phages show a burst of synthesis of both the *c*I repressor (26,000 molecular weight band) and *int* gene product (36,000 molecular weight band). This suggests that the inverted segment containing the *int* gene includes also its promoter for fast expression. Third, several of the major late protein bands (E, V and others) can be recognised in Fig. 2. None of these proteins is affected by the inverted segment. Fourth, a protein with a molecular weight of 31,000, designated X_1, is synthesised early after infection with λgt–λC but not after infection with λgt–λC′. This protein band is not present in infections with λ$^+$, nor has it been observed in a large number of phage mutants screened in our laboratory. We do not know the origin of this protein band nor why it is not synthesised in λgt–λC′. It may result from a genetic fusion in the construction of the phage, probably at the R1–2 site.

These results indicate that fast expression of the *int* gene originates at a promoter located within the *att–exo* region and is independent of transcription from P_L.

I thank Dr Ronald Davis for phage strains; Dr A. Oppenheim, Mrs N. Katzir, Mrs N. Kass and Miss S. Abergail for help. This work was supported by a contract with the Abteilung für Molekular Genetik der Gesellschaft für Strahlen und Umweltforschung Göttingen and the United States–Israel Binational Science Foundation.

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reviews

Mustard seed of mystery

Michael W. Ovenden

The Sirius Mystery. By Robert K. G. Temple. Pp. viii+290+21 plates. (Sidgwick and Jackson: London, 1976.) £6.95.

THE Dogon of west Mali are an artistic people, whose sculptures had a strong influence on Picasso and Braque. Two French anthropologists, Marcel Griaule and Germaine Dieterlen, lived for a time with the Dogon, and (by special permission of the priests assembled) Marcel Griaule was initiated into some of their most secret knowledge.

There is a strong astronomical flavour to some of this esoteric tradition. For example, the Dogon know of the four bright satellites of Jupiter (which are, however, just visible to an acute and attentive human naked eye). But the Dogon also describe a star companion to Sirius. They name this star after the smallest seed known to them, translated by Griaule to its Latin form *Digitaria*. *Digitaria*, say the Dogon, is the smallest and heaviest thing in the sky, and it moves around Sirius with a period of 50 yr. Now Sirius B is a white dwarf—and its period about Sirius is given (Aitken, 1932) as 50.04 ± 0.09 yr. Yet the visual magnitude of Sirius B is about 8.7; Sirius B is totally invisible to even the most acute human eye. This is the Sirius Mystery—the starting-point for Robert Temple's fascinating, frustrating and ultimately sad book.

In approaching the mystery with that open-minded scepticism which is the hallmark of (ideal) science, we might wonder whether the reporting of the tradition has been influenced by the astronomical knowledge of the reporters. The internal evidence is against this. The anthropologists seemed quite unaware of the extraordinary nature of their discovery, or how accurately the Dogon drawings of the path of *Digitaria* match the apparent (projected) orbit of Sirius B about Sirius. Can we postulate some astronomically-minded colonial administrator or seeker of artefacts who put, for the Dogon, a twentieth-century gloss on their ancestral astral traditions? Unlikely—for the behaviour of *Digitaria* plays a significant, albeit obscure, part in the chronology of religious ceremonies that clearly go back several hundred years, to long before Alvan Clark 'discovered' Sirius B in 1862 (using an 18.5-inch refracting telescope). And how could our hypothetical astronomer explain Dogon statements about the Sirius system that are *not* part of present astronomical understanding—such as the 'fact' that *Digitaria* is brighter when it is nearer to Sirius, a proximity effect which is neither observed now nor explicable by present ideas, in such a wide system as that of Sirius.

The four Dogon priests who revealed the Sirius traditions to anthropologists (Top : *Manda D'Orosongo and Innekouzou;* bottom : *Ongnonlou and Yébéné*)

How came the Dogon on such strange knowledge? Robert Temple takes the view that the Dogon traditions are part of an earlier tradition from elsewhere. He devotes much space to the origins of the Dogon, whom he links with the Garamantes of Herodotus, and ultimately with the Argonauts. Maybe! But to explain the survival with the Dogon of ancient tradition we need only recall that, in the sixteenth century, at Timbuktu in Mali, there flourished a leading university of the Muslim world. Through Timbuktu would have flowed the traditions of Greece, Egypt and Sumer.

At this point, Robert Temple sets off on a scramble through classical literature and Egyptian cosmology, seeking hints of the same Sirius mythology. There is much to be enjoyed here—especially the byways of Proclus and the neoplatonists, whose influence on the development of modern science is only just being realised. But (for example) to treat every reference to the number '50', from the oars of the Argonauts to the weight of the armour of Gilgamesh (50 minas) as sublime references to the period of Sirius B, fails to carry conviction with me. The tradition is supposed to be esoteric—but if it is as esoteric as that, we cannot unscramble it.

All this simply relocates the mystery in time and place; it is still a mystery. But not to the Dogon. In their mythology, *Digitaria* and the Sirius system are sacred, because from these came the Nommo—fish-like creatures who instructed mankind in the arts of civilisation. Of course, Robert Temple sees at once a connection between the Nommo of the Dogon and Oannes and the Annedotus of the Chaldeans; he kindly reprints for us in English translation the accounts of Oannes from Berossus *et al.* Oannes came from the Erythraean Sea, not from Sirius—so far so good. The author goes on to suppose, however, these myths to be literal statements of historical truth. Is this reasonable? The answer is—we simply don't know. Robert Temple is not the first non-astronomer to be misled by calculations of the 'probability' of life on other worlds, which are simply the prejudices of the respective writers dressed up in a spurious numerical precision. Certainly the idea of intelligent visitors from space is not *absurd*. How much evidence one would require before embracing the hypothesis tells more about one's psychology than it does about the historical problem.

This is a fascinating book because the nugget of mystery that the author has mined and polished is from a pure vein. The book is frustrating because the arguments are loose, and much of what the author has written is only marginally connected with his theme. A shorter, tighter book would have made more impact.

Ultimately, the book is sad for two reasons. In the first place, Robert Temple attempts to dissociate himself from ". . . the people who will enthusiastically receive my researches with open arms (but who are) the sort of people one least wants to be classed with". The author brings to his work a scholarship in oriental studies, and a belief in it that is transparently honest. The book should be taken seriously. Nevertheless, when all has been said and done, he (like others before him) is building a pyramid of speculation on a mustard seed of mystery. Such speculation can be judged only by its fruits. How often, since first learning of these ideas, have I been able to say, in my historical reading, "ah yes, that fits in so well." The answer is, not once; but perhaps I have been reading the wrong works.

This book is sad to me also in a different and deeper way. By taking myth only as history, we demythologise it. Myths (so I believe) express truths about the human predicament that cannot be expressed except in mythopoetic languages, in whose very ambiguity lies a richness of understanding. To take myth as history is to reduce it. It may be so. But I would rather my gods danced still on Mount Olympus than merely to have leased a country house in one of the more desirable subdivisions of Thessalia. ☐

Michael Ovenden is Professor of Astronomy in the University of British Columbia, Vancouver, Canada.

Vision in children

The Visual World of the Child. By Eliane Vurpillot. Pp. 372. (Allen and Unwin: London, April 1976.) £9.50.

If 4-yr-old children are presented with two panels bearing a square and a circle, they can learn to select the square consistently to obtain delivery of a sweet. If the experimenter then makes the sweet contingent on their pressing the circle, they normally take longer to learn this 'discrimination reversal' than they took to learn the original task. There is a story that as reversal training was about to begin one experimenter accidentally knocked over the apparatus that presented the stimuli and rewards: he restored it to an upright position, remarking that he hoped it was not broken. The child learned the reversal in a single trial. The story illustrates the extent to which children's behaviour may be governed by their expectations of how a piece of apparatus will behave and of how experimenters themselves are likely to behave. Such considerations have largely been ignored by the long list of psychologists whose work is summarised in *The Visual World of the Child.*

Disappointingly few secure or interesting conclusions emerge from Mademoiselle Vurpillot's catalogue raisonée of experiments. Many of the theoretical issues raised are in any case so ill formulated or so trivial as to preclude the possibility of interesting answers. A question of the former sort is whether the child proceeds from 'syncretic' to 'analytic' vision—no-one knows; one of the latter sort is whether a child's eye movements become more systematic with age—they do. Research on visual development, particularly in Europe, still suffers from Piaget's obfuscations and some of the master's vague concepts, like 'centration' and 'assimilation' have proved singularly unhelpful when applied to perception, but are unfortunately still taken seriously. Moreover, the plethora of contradictory experimental results in this area stems in part from the difficulty in replicating many of his experiments..

Although the book comes with the good psychologist's seal of approval, provided in an encomium written by Professor Jerome Bruner, it will be useful mainly to those who would like some signposts through the morass of the European literature on vision in children. **N. S. Sutherland**

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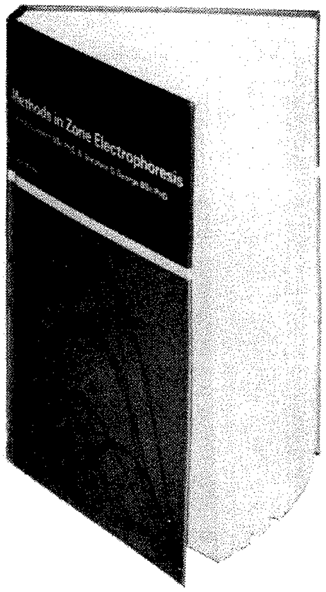

Methods in zone electrophoresis

J. R. Sargent, BSc, PhD, and
S. G. George, BSc, PhD
3rd edition, 1975, considerably enlarged and revised A5 (210 X 148mm) cloth cover.

The continued growth and improvements in electrophoretic methods during the few years since the second edition of this book was published has made it necessary to bring it up to date.

The 3rd edition comprises sevent chapters. After an outline of general theory, high and low voltage electrophoresis are described, together with chapters on cellulose, thin layer, starch, agar (including immunoelectrophoresis) and polyacrylamide media. The chapter on column electrophoresis includes data on supports, density gradients, isoelectric focusing and isotachoelectrophoresis.

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Excited states

The Excited State in Chemical Physics. Vol. 28. Edited by J. Wm McGowan. (Advances in Chemical Physics.) Pp. x+492. (Wiley/Interscience New York and London, October 1975) £13 75

THIS recent addition to this well established series is planned as the first of two volumes intended to cover the properties and reactions of excited states of atoms and simple molecules. In it several experts critically review various aspects of the physics and chemistry of mainly neutral excited species. The first and longest chapter, by I. W. A. Smith, on the production of excited species in simple chemical reactions, provides a useful introduction, with almost 500 references, to molecular beam studies of reaction dynamics and to spectroscopic methods for studying excited species. R. C Amme discusses equally well theory and experimental techniques concerning vibrational and rotational excitation in gaseous collisions, and J. J. Kaufman clearly demonstrates, in her contribution, the usefulness of potential energy surface considerations in elucidating the detailed mechanisms for reactions which involve electronically excited states.

There is a chapter entitled 'Theory of Non-adiabatic Collision Processes including Excited Alkali Atoms' by E. E Nikitin, whereas the emphasis is mainly experimental in the article on 'Sensitized Fluorescence and Quenching' by L Krause. The final two short chapters are interesting accounts of applications to lasers by R. H Bullis and of 'Excitation and De-excitation Processes Relevant to the Upper Atmosphere' by J W. McGowan, R. H. Kummler and F. R Gilmore.

It is a pity that delays in publication dates have caused some of the chapters first written in 1969 to be redrafted later and to include supplements written in 1974 In rapidly expanding areas such as those discussed, this kind of delay means that the reviews and literature surveys become rapidly outdated Although this is not of supreme importance for the non-specialist for whom this volume is mainly intended, it is nevertheless a disadvantage

F. Wilkinson

Cell membranes

The Molecular Biology of Cell Membranes By Peter J Quinn. Pp x+229. (Macmillan: London and Basingstoke, 1976.) Hard covers £8.95: paperback £3.95.

THIS book has developed partly from a course given to undergraduates and is aimed at "all students of the biological sciences who have completed a first course in cell biology or biochemistry" and "teachers and research workers in the field" I suspect that it will prove more useful to the latter group. In five chapters it covers Characterisation, Physical Structure and Molecular Organisation of Membranes, Membrane Permeability and Transport and Regulation of Cellular Processes by Membranes Its particular emphasis, except in chapters 1 and 5, is on biophysical studies of model membrane systems and their relevance to the function of biological membranes. Sometimes this emphasis is excessive and one wonders whether Molecular Biology is an appropriate title—for example, we are told how liposomes and asymmetric black lipid films are made, but not how to isolate biological membranes Biology only dominates chapter 5, and plants and prokaryotes hardly get a mention

The format is largely textual, with only a minority of the illustrations of an interpretive nature, and most undergraduates will probably learn relatively little from the frequent reproduction, without full interpretations, of original research data The text itself is quite good, although it would be better if it was considerably more concise and carefully organised There is a failure to cross reference apparently contradictory statements (for example, chapters 1 and 2 disagree on the significance of the amino acid compositions of intrinsic proteins, and some qualifying comment should accompany the separate statements that endoplasmic reticulum possesses 15 polypeptides and 75 enzymes). Some items are idiosyncratic, such as the description of peptide ionophores as proteins and the description of erythrocyte proteins mainly by reference to a single paper by Tanner and Boxer Others are wrong—for example, gangliosides without sialic acid, the equating of glyceryl ethers with plasmalogens and of polypeptides separated by electrophoresis in SDS with proteins, secretion of digestive enzymes by pancreatic α cells, and the statement that the permeability of lipid bilayers to water is 8–9 times greater than to sugars and ions All in all, the book may be useful to the well-informed and critical teacher looking for up-to-date and detailed information on model membranes, but it is neither balanced enough nor accurate enough for use as a main undergraduate text on biological membranes.

Robert H. Michell

Plumbing the depths

Underwater Research. Edited by E. A. Drew, J. N. Lythgoe and J. D. Woods. Pp. viii+430. (Academic: London and New York, March 1976.) £14, $34.75.

ALTHOUGH so much less spectacular, the gradual conquest of the ocean depths has certainly been more immediately productive and at vastly lower cost than the conquest of outer space. What began largely to further biological observation has assumed impressive dimensions in the present search for oil, to be followed by that for mineral reserves.

This composite book, containing no less than 25 contributions, gives a first impression of over-diversity but this is in some measure alleviated when it is found that the first three papers represent almost half the contents. These deal with such fundamental matters as communication between, and hearing in, divers (Hollien and Rothman; Hollien and Feinstein) and problems of underwater vision (Cocking). These are written by active workers and bring us up to date (at the time of writing) with the progress in what are rapidly developing technologies. Increasing improvement here is essential if divers are adequately to co-operate and to observe in this alien environment.

Most of the remaining articles were originally contributed to the annual symposia of the Underwater Association The first are concerned with aspects of underwater physiology such as the effects of narcosis and the measurement of respiration at high pressures. There is concern with underwater habitats, with surveying on the seabed and with quantitative estimations of underwater benthos.

The last lead on to straight zoological communications dealing with the crown-of-thorns starfish and the British cup coral, *Caryophyllia.* Botanists will find interest in work on submerged freshwater macrophytes and on the growth and physiology of *Posidonia* at Malta and of *Laminaria* in Scotland. I was particularly interested in R. K. Trench's admirably brief review of calcification in reef-bulding corals, although, with delays imposed by this form of publication, no longer quite up to date The volume ends with an account of archaeological evidence for sealevel changes in the eastern Mediterranean.

One sees how wide the net has been cast; there is much here to interest all concerned with underwater activities and the book can be recommended for inclusion in libraries that are available to them.

C. M. Yonge

Taxonomy and medical aspects of amoebae

Pathogenic and Non-Pathogenic Amoebae By B N. Singh. Pp. ix+235. (Macmillan. London and Basingstoke, October 1975.) £15.

Dr Singh's book is divided into two sections—'Aerobic' and 'Anaerobic'. 'Anaerobic amoebae' is devoted to a full and useful review of work (much of it carried out in India) on the cultural requirements and pathogenicity of the dysentery amoeba. The problem of encystment is dwelt on, and methods of killing the cysts while still within the patient are discussed Encystment and excystment factors are examined, mainly by using aerobic amoebae as model organisms 'Aerobic amoebae' covers the amoebae of soil and water, and types of particular interest, taxonomically and medically are described in detail.

Confusion may arise in many minds when the taxonomic assignments of Singh and of the occidental authority, F. C. Page, are compared. Page considers, justifiably, I think, that Singh's *Hartmannella* comprises the two genera *Hartmannella* and *Acanthamoeba.* All pathogenic strains of *Hartmannella* (Singh) so far isolated would fit into Page's *Acanthamoeba.* There is a further conflict between Singh and Page over the existence of the genus *Didascalus* which Page would incorporate in the genus *Naegleria. Didascalus* as described by Singh has a 'promitotic' cell division without interzonal body formation, and is capable, with some difficulty, of producing a flagellate stage. *Naegleria,* according to Singh, invariably produces interzonal bodies during nuclear division; but, according to Page, this is not a consistent character In the reviewer's experience, although the 'promitotic' division of *Naegleria* does not invariably reveal interzonal bodies, it generally does, and it seems sensible to separate *Didascalus* which never does, from *Naegleria* which generally has the interzonal bodies and is readily capable of forming flagellates.

A third point of conflict is linked to the preceding one, and is concerned with the nomenclature of amoebae with 'promitotic' division which do not produce a flagellate stage. Decision on this point is much more confusing, as it depends on interpretation of old descriptions in the literature, which are not even certainly of pure cultures. It is however, sufficient to say that the genus termed *Schizopyrenus* by Singh is in all respects that termed *Vahlkampfia* by Page.

There is a fourth point in which Singh differs from what seems to be current terminology. He argues that the pathogenic HB1 strain of *Naegleria* he initially studied is the same species as that isolated and studied by the Australian workers (This is supported by independent work) R. F Carter named the Australian organism *N. fowleri* in a publication slightly predating the Indian workers' description and naming of HBI as *N.aerobia.* After a rather tortuous argument, Singh claims that *N aerobia* has priority over *N fowleri.*

The foregoing points of taxonomic confusion serve to emphasise the need for the objective taxonomic criteria of a biochemical and immunological nature which are in the course of emerging in this rapidly expanding field.

The book is well produced and easy to read. It should form a useful introduction to the subject for the student or the research worker

D. C. Warhurst

Immunosuppressive agents

The Mode of Action of Immunosuppressive Agents. (North-Holland Research Monographs: Frontiers of Biology, Vol. 41.) By Jean Francois Bach. Pp. xviii+379 (North-Holland· Amsterdam and Oxord; American Elsevier: New York, 1975). Dfl. 98; $40.95.

This book is intended for the clinical immunologist who is involved with the use of immunosuppressive agents in man. Rather than review all the data related to immunosuppressive agents, for which there is admittedly scattered and incomplete information, the author has chosen to discuss four classes of agents in detail: corticosteroids (hydrocortisone and its derivatives), thiopurines (6-mercaptopurine and azathioprine), alkylating agents (cyclophosphamide) and anti-lymphocyte serum

In the first chapter (general introduction) several important considerations related to immunosuppressive agents are discussed. This is a good beginning and provides the proper perspective for the subsequent chapters The problems of definition of an immunosuppressive agent, anti-inflammatory effect, therapeutic index, drug evaluation and the experimental approaches to investigating a given drug are reasonably well covered

The outline of the subsequent chapters are quite good and begin with the known chemistry of the agents, their pharmacology, toxicity, and so on, and proceed through a review of the effects of the agents on various aspects of the immune response, including clinical trials. Unfortunately, the promise provided by the first chapter and the logic of the outline of the subsequent chapters is not fulfilled by the text material.

Often the summarised reports represent a cataloguing of data and not enough critical evaluation is summarised by the author. The result will tend to confuse the new reader. A more serious objection is the lack at times of emphasising important findings that may have exciting implications. For example, cyclophosphamide may prevent allergic encephalomyelitis or thyroiditis but it will also produce a 'tolerant' state. The latter aspect is not discussed nor even noted.

Some of the review is quite outdated (for example, metabolism of cyclophosphamide) but more seriously there are a number of errors in reporting the literature as well as uncritical reporting. On p184, for instance, the author states that bone marrow grafts must be carried out 24 h after cyclophosphamide injection, because of the effects of residual cyclophosphamide on engrafted cells. This is true for the rat but certainly not true for the mouse. On p203, under the heading of tumor immunity, it is stated that cyclophosphamide may favour the development of L1210 leukaemia in C57BL mice. What is meant most certainly is that L1210 can be transplanted to a C57BL/6 mouse given cyclophosphamide. What does this have to do with tumour immunity, as this is an allograft!

The reference quoted is wrong and the data not accurately reported in several places in the chapter on alkylating agents

Chapter 6 contains concluding remarks and guidelines for the clinical use of immunosuppressive agents. It is also disappointing, particularly in the choice of dosage and monitoring of the effects of a given agent on a disease process In treating a disease process it would seem wisest to decide what clinical effects one desires and then treat the disease with doses of agents designed to give acceptable clinical toxicity. The acceptable toxicity, of course, will be dictated by the severity of the disease being treated The author does not emphasise this philosophy but instead discusses *in vitro* monitoring of immunological function.

In view of the serious criticisms listed above, I cannot recommend this book to those interested in immunosuppression or immunology

George W. Santos

obituary

Carl Peter Henrik Dam died in April 1976. He was born at Copenhagen in 1895 and educated there. He went to Graz (1925) to be trained in microanalysis under Pregl and later worked with R. Schoenheimer at Freiburg (1932–33) and with P. Karrer at Zurich. He always tried to keep up his skill as a chemist while steadily widening his biological interests

Early in his career (1929) Dam made the first observations that led by stages to the discovery of vitamin K and other 'bioquinones' and towards the end of his career his friends delighted to remind him "How far that little candle throws its beams".

He began by testing a claim that chicks could not thrive on an almost sterol-free diet. Some of his birds unexpectedly displayed haemorrhages under the skin, in muscles or other tissues, and the coagulation of their blood was much retarded McFarlane in Toronto confirmed this. Dam's research was delayed by his visit to Freiburg, but on his return he and Schönheyder found that the chick symptoms were not due to sterol deprivation but to a dietary lack of anti-haemorrhagic vitamin from plants, first designated vitamin K and now known as phylloquinone. A chick bioassay was used to monitor the separation of lipid factors from alfalfa. Dam joined forces with Karrer in isolating and characterising the vitamin which was recognised (both at Zurich and in the USA) as 2-methyl-3-phytyl-1,4-naphthoquinone. With the methods then available this was a considerable feat Phyloquinone is, quantitatively, a minor constituent of the quinones present in chloroplasts, and although the bio-assay successfully led to the vitamin, the more plentiful quinone congeners were missed

It was found in the USA that putrefaction of ether-extracted fish meal changed it from a very poor to a very potent source of vitamin K activity The formation of vitamin K by bacteria was followed up by Almquist and Stokstad and by Doisy's group. In addition to isolating phylloquinone they obtained a second vitamin K (at first known as vitamin K_2 but now as a menaquinone). The new form was a 2-methyl-3-polyprenyl-1,4-naphthoquinone which later turned out to be one member of a large family of menaquinones denoted MK-*n*, where *n* is the number of prenyl (C_5H_8) groups in the polyprenyl sidechain.

Vitamin K activity was also found in a number of simpler but related substances (for example 2-methyl-1,4-naphthoquinone, or menadione, and numerous esters of derived quinols). These substances were seen as provitamins K A new facet to the larger problem was Link's isolation and characterisation from spoiled sweet clover hay of dicoumarol, a specific vitamin K antagonist. The number of antagonists is now large, some of the best-known being dicoumarol, Warfarin, phthiocol, sulphaquinoxaline and actinomycin D.

The coagulation of blood is envisaged as a complex but ordered succession of processes, and at least four of the many factors (prothrombin, proconvertin, Christmas factor and Stuart factor) are known to be dependent on vitamin K Of these the deficient chick lacks only the Christmas factor. Dam's group studied neonatal bleeding disorders, and advocated the administration of vitamin K to mothers a few hours before parturition. They also noticed that cows' milk is much richer in vitamin K activity than human milk.

In 1940 Dam went on a lecture tour in the USA and Canada and then became a Senior Research Associate at the University of Rochester (1924–45) returning to Denmark in 1946. In 1943 Dam and E. A. Doisy shared the Nobel Prize for Physiology and Medicine. While still in the USA Dam had been appointed to the Chair of Biochemistry and Nutrition at the Copenhagen Polytechnic Institute which he held until his retirement in 1965. He also played a leading part in the work of the Danish Fat Research Institute (1956–63). He led a research school and published nearly 300 papers mostly concerned with cholesterol, vitamin E and vitamin K. His review articles were models of fairness and acumen as well as deep scholarship

In 1966 the 4th Roche International Symposium was held at Elsinore in his honour. It was entitled 'Recent Advances in Research on Vitamins K and Related Quinones (Ubiquinones or Coenzymes Q and Plastoquinones)'. By that time the chemical diversity within the major families or bioquinones was evident and the central biological roles of quinones, quinols and chromanols had become as acceptable as they were surprising.

The 'prothrombin' synthesised by cattle given dicoumarol is not effective in blood coagulation and fails to bind calcium ions or phospholipids Ten defined positions on the sequence 1–42 are occupied by glutamyl residues, whereas in normal prothrombin all ten are carboxylated to a previously unknown γ-carboxyglutamic acid. The vitamin K-dependent step in the formation of prothrombin is a post-synthetic γ-carboxylation of a precursor made in liver Thanks to coalescing research from Denmark, Sweden, the USA and Britain, the principal role of vitamin K in the animal is perhaps now clear in the sense that what the vitamin does is established, if how it does it remains to be worked out.

At the symposium in his honour Dam was obviously gratified by developments arising from his original observations and it is very fitting that the recent work, entirely contemporary in its approach, should have come so near to rounding off his discovery.

Professor Dam travelled a good deal and was among the best known and most welcomed scientists in an ever-widening field.

R. A. Morton

announcements

Awards

The **William Bate Hardy Prize** of the Cambridge Philosophical Society has been awarded to **Dr F. Sanger, FRS,** of King's College for his distinguished work on the sequencing of DNA

The Société Française de Physique has made the following awards:

Le grand prix de physique Jean-Ricard to **Georges Slodzian** of the University of Paris-Sud (Orsay)

Le prix Robin to **Jacques Prentki** of the College de France

Le prix Ancel to **Gerard Jannink** of the Centre d'Etudes Nucléaires de Saclay

Le prix Aime-Cotton to **Michel Gaillard** of the University de Lyon

Le prix Paul-Langevin to **Gerard Toulouse** of the Laboratoire de Physique des Solides à Orsay

Le prix Esclangon to **A. Quinzer** of the Laboratoire de l'Accélérateur Linéaire d'Orsay

Le prix Foucault to **J.-P. Taran** of the Office national d'Études et de Recherches Aérospatiales.

International meetings

August 1–7, **High Speed Photography,** Toronto (Congress Secretariat, c/o Ontario Science Centre, 770 Don Mills Road, Don Mills, Ontario, Canada M3C 1T3).

August 2–3, **Rocky Mountain Spectroscopy Conference,** Denver (Ms Jean R. Gaines, 1012 4th St, Golden, Colorado 80401).

August 18–27, **Mathematical Geophysics,** Seeheim/Odenwald, FRG (Professor Karl Fuchs, Geophysikalisches Institut, Universität Karlsruhe, Hertz-Str. 16, 75 Karlsruhe, FRG).

August 19–27, **Entomology,** Washington DC (Dr Ernest C. Bay, Secretary General, XV International Congress of Entomology, PO Box 151, College Park, Maryland 20740).

August 22–27, **Liquid Crystals,** Kent, Ohio (Organising Committee, Liquid Crystal Institute, Kent State University, Kent, Ohio 44242).

August 22–28, **Transplantation,** New York City (Congress Secretariat, Felix T. Rapaport, New York University Medical Center, 560 First Avenue, New York, N.Y. 10016).

August 23–27, **Analytical Chemistry in the Exploration, Mining and Processing of Materials.** Johannesburg (The Conference Division (IUPAC Symposium), CSIR, PO Box 395, Pretoria, 0001, RSA).

August 27–29, **Forensic Toxicology,** Ghent (Professor A. Heyndrickx, Department of Toxicology, State University of Ghent, Hospitaalstraat 13, 9000 Ghent, Belgium).

August 29–September 3, **Invertebrate Pathology,** Kingston, Ontario (Dr Peter Faulkner, Department of Microbiology and Immunology, Queen's University, Kingston, Ontario, Canada K7L 3N6).

September 22–24, **Pressure Surges,** London (The Organising Secretary, 2nd ICPS, BHRA, Fluid Engineering, Cranfield, Bedford MK43 0AJ, UK).

Person to Person

The annual introductory course on tropical hygiene will be held at the Ross Institute this year from July 12–16. The course is designed for para-medical and non-medical people proceeding to the tropics, especially for those who may be responsible for the health of others, and is directed towards giving a comprehensive understanding of the prevention of tropical diseases caused by infection, insanitation and malnutrition. There is no fee for the course. Recognising its value, many companies and institutions with interests in the tropics pay the expenses of their staff during their stay in London, and it is hoped that this practice will become general. Contact: Ross Institute of Tropical Hygine, London School of Hygiene and Tropical Medicine, Keppel Street (Gower Street), London WC1E 7HT.

Professor A. K. Som of University of Malawi, Box 280, Zomba, Malawi, on sabbatical leave, wishes to rent a centrally heated furnished 2/3 bedroom house or flat in London from August '76 to Sept. '77. Please refer to Dr G. Leventhall, Physics Dept., Chelsea College, London.

There will be no charge for this service Send items (not more than 60 words) to Martin Goldman at the London office The section will include exchanges of accommodation, personal announcements and scientific queries We reserve the right to decline material submitted No commercial transactions

November 19, **X-Ray Diffraction,** Wembley (Abstracts by October 1 to Dr B. J Isherwood, GEC Hirst Research Centre, East Lane, Wembley, Middlesex HA9 7PP), (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

February 28–March 4, 1977, **Analytical Chemistry and Applied Spectroscopy,** Cleveland, Ohio (Deadline for abstracts: September 15), (John E. Graham, Program Chairman, Koppers Company, Inc., 440 College Park Drive, Monroeville, Pennsylvania 15146)

July 26–29, 1977, **Electron Transport/Molecular Solids,** Leeds (Preliminary offers of papers by October 1 to Professor G. J. Morgan, Department of Physics, University of Leeds, Leeds LS2 9JT, UK), (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

Reports and publications

Control of the Noise and Vibration Environment. By Professor P. Grootenhuis (Inaugural Lecture, 12 November 1974) Pp 15. £1 10. Complex Fluids in Engineering By Professor J R. A. Pearson (Inaugural Lecture, 3 June 1975) Pp. 37–444 90p (London Imperial College of Science and Technology, University of London, 1974 and 1975) [103

Philosophical Transactions of the Royal Society of London A· Mathematical and Physical Sciences Vol. 281, No. 1301 The Symmetry of Electron Diffraction Zone Axis Patterns By B F Buxton, J A Eades, J. W Steeds and G M. Rockham. Pp. 171–194 + plates 1–3. (London. The Royal Society, 1976) UK £1.75; Overseas £1 80 [113

Royal Observatory Annals. No. 10. Photoheliographic Results 1966 Pp 67. (Herstmonceux. Royal Greenwich Observatory, 1974.) [113

Energy Audits (Fuel Efficiency Booklet, No 1.) Pp 12 (London: Library, Department of Energy, Thames House South, Millbank, SW1, 1976.) *gratis.* [123

Bulletin of the British Museum (Natural History) Entomology Vol 32, No. 6. A Revision of the Genus *Ptychandra* (Lepidoptera· Numphalidae). By H J Banks, J D. Holloway and H S. Barlow Pp. 215–252 + 5 plates. (London British Museum (Natural History), 1976) £4 25 [123

Science and the Media–Report of a Study Group (British Association Publication 76/1.) Pp 27 (London British Association for the Advancement of Science, 1976.) 50p. [123

Male and Female Sterilization, Second edition Edited by R. L Kleinman. Pp 39. (London International Planned Parenthood Federation, 18/20 Lower Regent Street, SW1, 1976) 80p, $1.75. [153

Bulletin of the British Museum (Natural History). Botany. Vol. 5, No 5 Frank Ludlow (1885–1972) and the Ludlow-Sherriff Expeditions to Bhutan and South-Eastern Tibet of 1933–1950. By W T Stearn. *Reliquiae Botanicae· Himalaicae* By Frank Ludlow Pp 241–289 + plates 30–36. £4 75 Entomology. Vol. 32, No. 5 An illustrated List of the Type-Specimens of the Heliconiinae (Lepidoptera: Nymphalidae) in the British Museum (Natural History) By P. C Ackery and R L. Smiles. Pp 171–214 + 39 plates. £10.70 Geology Vol 26, No 6: Dinoflagellate Cysts from the Brackesham Beds (Eocene) of the Isle of Wight, Southern England. By G. L. Eaton Pp 225–332 + 21 plates £11 60. (London British Museum (Natural History), 1976) [153

Bedfordshire Plant Atlas. By John G. Dony. Pp 132 (Luton Borough of Luton Museum and Art Gallery, 1976.) £3. [153

Register of Consulting Scientists, Contract Research Organizations and other Scientific and Technical Services. Third edition. Pp 137. (Stoke Poges, Slough. Hon. Registrar, Fulmer Research Institute, Ltd , 1976.) £7 + 50p. postage and packing. [153

The Association of Commonwealth Universities. Annual Report of the Council, 1974–75. Pp. 50 (London· The Association of Commonwealth Universities, 1976.) [163

Engineering Design Education, (A Report on the Current Education in Engineering Designers in Britain with Recommendations for Future Policies and Action) Pp 12. (London· Design Council, 1976) 50p [173

Natural Environment Research Council International Hydrological Decade Hydrological Research in the United Kingdom (1970–1975) Pp. vi + 180. (London Natural Environment Research Council, 1976.) [173

Computers and Chemistry, Vol. 1, No 1 Pp. 1–68 Annual Subscription Rates for 1976 For Libraries, Research Establishments and all other Multiple-Reader Institutions $60 (Oxford and New York: Pergamon Press, 1976) [183

Philosophical Transactions of the Royal Society of London B Biological Sciences. Vol. 274, No 929 The Structure and Function of Thoracic Expodites in the Larvae of the Lobster *Homarus gammarus* (L.) By D. M. Neil, D. L. Macmillan, R M Robertson and M S Laverack A Quantitative Analysis of Exopodite Beating in the Larvae of the Lobster *Homarus gammarus* (L) By D. L Macmillan, D. M. Neil and M S. Laverack. A Comparison of Beating Parameters in Larval and Post-Larval Locomotor Systems in the Lobster *Homarus gammarus* (L.). By M S Laverick, D L Macmillan and D M Neil. Pp. 53–99. UK £2 50, Overseas £2 60 Vol. 274, No 930 The Nervous System of *Loligo* II Suboesophageal Centres By J Z. Young, FRS Pp. 101–167 + plates 1–10. UK £5 30, Overseas £5 45. (London· The Royal Society, 1976) [193

Shirley Institute. Report and Accounts, 1974/1975 Pp 20. (Didsbury, Manchester· Shirley Institute, The Cotton Silk and Man-made Fibres Research Association, 1976) [193

Department of Trade. Report by the Department of Trade on the action taken to deal with the oil spilled as a result of the collision in the Dover Strait on 12 November 1975 between the M/T Olympic Alliance and HMS Achilles. Pp. 20. The Battle Against Oil Pollution at Sea Pp 12 (London Department of Trade Obtainable from The Marine Library, Sunley House, 90/93 High Holborn, London, WC1) [193

Review of the Water Industry in England and Wales a Consultative Document Pp 33 (London· Department of the Environment, 1976) [193

The British Library. Research and Development Reports Report No. 5233. British Library Working Party on Classification and Indexing Pp. 25. £2, $4. Report No. 5256· Inventory of Bibliographic Data Bases Produced in the UK Pp 82. £2 50, (Boston Spa, Wetherby: Publications Department, The British Library Lending Division, 1975 and 1976) [193

nature June 24, 1976

Fifty years on

NIT-PICKERS will point out that the Commonwealth Scientific and Industrial Research Organisation (CSIRO) is not fifty years old this week—the body founded in 1926 was the Council for Scientific and Industrial Research (CSIR), and the transition in name only came in 1949. This cannot be allowed to diminish the satisfaction that such a major scientific venture is still alive, well, and showing no obvious signs of senility. Elsewhere in this issue ten or so authors describe some of the aspects of CSIRO, past, present and future. (It is worth recording that we have noticed, not for the first time, that Australian scientists, when commissioned to contribute to *Nature* not only say yes without grumbling about how unreasonable the demands are and how immensely busy they find themselves all of a sudden, but also actually deliver the material requested . . . in good condition . . . ahead of schedule.)

One thing that strikes the visitor to Australia is the public enthusiasm which still prevails for science and technology. This enthusiasm is not, strangely, converted into an unusually large allocation of funds to R & D activities—little over 1% of the GNP goes that way. But of this figure, government's share is, in the words of the 1974 OECD Examiner's Report on Science and Technology in Australia, 'exceptionally high'. This report goes on to note that 'the total resources of CSIRO for research and development are not much less than those of all universities taken together, and greater than the R and D effort of secondary industry'. Under these circumstances it is inevitable that CSIRO should be the subject of endless questioning, criticism and attack. It is a large target and many of the things people say about it are probably true and mutually contradictory. But what the OECD Examiners, with no axe to grind, said, is worth looking at again, if only because, following their report, it seemed as if there was widespread agreement that their comments had been sensible, and deserved serious attention.

CSIRO, they said, may be big and sometimes seem monolithic, but the worst thing would be to split up its more than thirty divisions and distribute them amongst government departments. Within the past year we have seen encouraging signs that the scientific community can be mobilised against such piecemeal dismemberment.

The imbalance between CSIRO and universities, they said, seemed to be too large; but the balance should be redressed by strengthening university research rather than by weakening CSIRO. There is little expansion still left in the Australian university system, but probably what the examiners were thinking more of was the contracting out of more CSIRO-type pure research which has traditionally been done in house—maybe making room within the organisation for more applied research, where the future probably lies for CSIRO.

The examiners further noted that the structure of CSIRO did not necesarily made interdisciplinary research easy, and one may note parenthetically that this could be the reason why the organisation has been rather slow off the mark in employing social scientists. There are encouraging signs now, however, of new flexibility in the management and if this cuts down the previous high degree of autonomy of division chiefs, it is probable that most of them are happier in the new mode of having to talk to each other.

Finally, perhaps the weakest aspect of Australian science, and CSIRO is no exception, is the industrial dimension. It is by no means clear that the blame for this can be put on remote professors or on too-single-minded division chiefs. Australian industry (or secondary industry as it is often called to distinguish it from agriculture and mining) is very frequently controlled from outside the country. The bright idea is sent over from some industrial laboratory in Europe or North America, and all the fellows down-under are expected to do is implement it. This has made for a difficult climate for industrial research and the problem is not easily soluble, even in the short term.

The founders of CSIR much admired the British Department of Scientific and Industrial Research but added a research spirit more akin to that of a university. Australia has profited greatly by this is the past fifty years, and the rest of the world has things to learn from CSIRO. Not least is the way in which career structure is founded on ability, not on length of service. In these days of obsession with job security the UK might turn to Australia, fifty years on, to learn one or two things about how to employ scientists. It would probably turn out to be a sobering exercise. □

Genetic manipulation to be patented?

A patent application governing commercial uses of recombinant DNA techniques has been filed in the United States. Colin Norman reports.

WHILE the scientific community has been loudly debating the potential hazards and benefits associated with a new technique for manipulating genes in living organisms, Stanford University and the University of California have been quietly trying to patent the technique in the United States. Rumours about the patent application, which apparently has been pending for at least 18 months, surfaced at a scientific symposium held at the Massachusetts Institute of Technology earlier this month; they were confirmed by university officials last week.

Though the people concerned with the patent application are reluctant to discuss it in detail while the matter is pending, the patent is understood to be worded broadly enough to cover commercial uses of any method of transplanting genes from one organism into another. The patent, if awarded, would not affect research uses of the technique, and it would apply only in the United States.

The basis for the application is research published in 1973 and 1974 by groups led by Stanley Cohen at Stanford and Herbert Boyer at the University of California, San Francisco. Their efforts led to the first demonstration that genes can be snipped from the DNA of virtually any organism and spliced into a bacterial plasmid (a ring of DNA which reproduces inside a bacterium independent of the bacterium's chromosomes). The key part of their research showed that the modified plasmid could be reinserted into a bacterium so that the transplanted, 'foreign' genes are copied each time the bacterium reproduces. It is understood that the patent application covers the process for constructing hybrid DNA molecules capable of self-reproduction, which means that, if awarded, it would also apply to transplanting genes into viruses and bacteriophages.

There has been speculation that the technique might, eventually, lead to such commercial uses as the insertion into bacteria of genes capable of producing pharmaceutical products, such as insulin, so that specially engineered bacteria would be capable of secreting expensive drugs. Another speculative commercial application would be to graft genes capable of fixing nitrogen into crop plants such as wheat, to produce a new variety capable of synthesising its own nitrogen fertilisers from the atmosphere. Such uses of the technique would be covered by the patent application.

Discussion of the patent application arose at a symposium on genetic manipulation held by Miles Laboratories at MIT on June 8–10. Noting that there have been persistent rumours that somebody is trying to patent the technique, a speaker asked whether any participants could shed some light on the matter. Cohen, who was present at the meeting, confirmed that Stanford and the University of California are looking into the possibility of taking out a patent, and he emphasised that neither he nor any of the other researchers involved would benefit financially if it were awarded.

A patent application can be filed in the United States up to a year after the information on which it is based has been made public. The first paper by Cohen and Boyer's groups was published in November, 1973, which indicates that the application should have been filed before November 1974. Asked last week whether that assumption is correct, a Stanford patent officer replied that "it would be reasonable to assume that we were prudent in filing our application". In some countries, such as Great Britain, a patent must be applied for before any public disclosure is made. Britain's National Research and Development Corporation initially looked into the possibility of seeking a patent on genetic manipulation techniques developed by Kenneth and Noreen Murray at Edinburgh University, but dropped the idea because of prior disclosure.

When the matter was raised at the Miles symposium, two concerns were discussed. First, some scientists were worried that the patent, if awarded, might interfere with research. And second, it was suggested that it may force some commercial concerns to seek a less safe, but patent-free, genetic manipulation technique. Neither concern seems to be valid, however, and in fact the patent, if awarded, could have some safety benefits.

As for the research implications, the patent would apply only to commercial use of the technique—it would not cover either academic or industrial research uses. Moreover, asked whether it may hold up beneficial applications of the technique, a Stanford official argued that royalties derived from the patent would be "reasonable" and would not limit its use.

As far as safety implications are concerned, the application seems to cover the key steps in the genetic manipulation process and thus, unless it is drastically narrowed by the US Patent Office, it would be difficult to see how a different patent-free process could be developed. If the patent is awarded, Cohen suggested at the Miles symposium that Stanford and the University of California could insist that commercial users of the process be required to sign an undertaking to abide by safety guidelines laid down by the National Institutes of Health (NIH).

NIH is due to issue guidelines on June 23 governing genetic manipulation research which it supports. At a meeting earlier this month, NIH Director Donald Frederickson briefed a number of industry officials on the guidelines, and though they met with general support, some drug company representatives expressed reservations about one or two provisions. The NIH guidelines, moreover, would not be binding on industry, since they would apply only to NIH-sponsored research. The patent may, therefore, provide a means of extending the coverage of the guidelines.

Cohen and Boyer's work was supported by the National Institutes of Health, but the federal government is unlikely to have a stake in the patent. Stanford has a standing agreement with the Department of Health, Education and Welfare (HEW) which gives the university patent rights on inventions produced from research supported by HEW grants, unless the project has been exempted from the agreement. According to an HEW official, the genetic manipulation studies were not exempted. Stanford patent officers have, however, been discussing the application with federal officials, particularly as regards establishing licensing arrangements if the patent is awarded. ☐

Pakistan's ambitious nuclear power programme

from Azim Kidwai, Karachi

TWENTY-FOUR nuclear power plants operational in a developing country by the end of the century seems too ambitious a programme in the present global context, but that is the plan at the drawing-board stage in Pakistan. Work on a second nuclear plant, at Chashma Barrage, on the river Indus, 150 miles south of Islamabad, starts in a few months' time. The project is estimated to cost Rupees 527 crores (527 million dollars). The 137-MW nuclear power plant at Karachi has been in commercial operation for the past five years. The Chairman of the Pakistan Atomic Energy Commission (PAEC), Mr Munir Ahmad Khan unfolded the revised crash plan for 24 nuclear power plants recently, in the wake of a bilateral agreement with France that includes the offer by the French of a nuclear reprocessing plant (cost 150 million dollars) for Pakistan.

The rationale for such an unorthodox accelerated nuclear power programme can perhaps be found in the poor energy resources of the country, coupled with the present very low *per capita* electrical energy consumption.

The known fuel resources in Pakistan have been estimated to be only 13 tons coal equivalent (TCE) *per capita*. The figure presents a dismal picture when viewed against the world average *per capita* resources of 1,200 TCE. Similarly, the *per capita* electrical power consumption in Pakistan at present stands at 140 kWh as against 1,360 kWh world average, which has been extrapolated to be 6,500 kWh by the end of the century.

The requirements at the close of the century have been estimated at 27,000 MW (current production is only 2,570 MW, and the huge Tarbela Dam will soon add another 2,000 MW). Hydropower and gas—the only two energy sources of consequence in Pakistan — could only supply 13,500 MW. There seems not much of an option left, since planning on imports of huge quantities of oil would be hazardous. The main argument of PAEC, though, is that "the nuclear power plant's fuelling would be much cheaper than those of oil fired plants. The generation cost of a nuclear power plant would be 33% cheaper than that of a conventional power plant". In the long run, seen against the recent oil crisis, the argument appears to have validity even taking the higher capital costs of nuclear power plants into consideration.

All this planning on nuclear power may undergo revision if oil is struck in large quantities in Pakistan. Tremendous efforts are on the way as the 1976-77 budget on oil exploration at Rupees 42 crores (42 million dollars) testifies. The allocation has been more than doubled as compared to the figures in the current year. But without oil, the main plank of energy strategy in Pakistan will have to be nuclear because of the good deposits of uranium discovered recently in Pakistan and soon to be exploited. Concerted efforts to locate more uranium deposits also continue. Some of the areas have also been found with sizeable extents of thorium-bearing mineralisation—this fits into Pakistani nuclear think-tank schemes which envisage branching off to thorium.

One nuclear plant each year, on average, is planned after the early 1980s. The scheme includes dual-purpose nuclear plants as well. For instance, feasibility studies on such a dual-purpose nuclear plant for Karachi were mounted some time back in which the city could add to its grid another 400 MWe by the early 1980s and could also augment its water supply by 100 million gallons of sweet water from the sea daily.

Such a vast programme of nuclear power development calls for considerable manpower with sophisticated technical know-how. The PAEC has been training a large number of scientists and engineers for the last decade to meet the present situation. There are already some 350 scientists and engineers trained abroad on the rolls of the PAEC and a large number have been or are being trained at the Reactor School at Nilore near Islamabad Another big nuclear power training centre which will cost Rupees 2.5 crores is being planned. Based at Karachi, and designed to train about 200 persons including technicians and operators annually, the centre would fill the gap on the technical manpower requirements of the PAEC in the coming years.

USSR's polar research

from Vera Rich

ARCTIC and Antarctic research has long been a major field of Soviet research Indeed, the establishment of the first *Severnyi Polyus* (North Pole) drifting ice-floe research station and the forcing of the North East Passage by an icebreaker in the 1930's had at that time much of the prestige value now associated with the space programme. This sense of the spectacular is still sometimes apparent in the announcement of new Soviet Arctic projects.

The "Polar Experiment North-76" from April to July this year, for example, was announced as the "largest comprehensive expedition in the history of Arctic exploration", involving ten research vessels (including the flagship *Professor Vize*), aircraft, and upper atmosphere probe rockets. According to Professor A. Treshnikov, Director of the Arctic and Antarctic Institute (Leningrad), the principal aim of the expedition is to calculate the thermal balance of the Arctic icecap simultaneously on land, in the ocean, in the atmosphere and on the drift-ice (including observations at the North Pole itself). The data gathered during the 100-day expedition will be used to formulate mathematical models of hydrological and meteorological processes for use in weather forecasting. The expedition is envisaged as the first in a series to take place at one- and two-yearly intervals as part of a programme sponsored by the World Meteorological Organization.

Results of the previous voyage of the *Professor Vize* are meanwhile being processed. That expedition, during the Antarctic summer of 1975–76, produced data indicating that the Antarctic circumpolar current is "equal in flow to six Gulf Streams". The research vessel *Dmitrii Mendeleev* also visited the Antarctic during the same summer on its first biological expedition. According to the expedition leader, Dr L. A. Ponomareva, its programme included research into the fauna of the ocean bed, the sampling of water from the bottom layers of the Macquarie complex, study of the Macquarie and Hjort trenches, the collection of sedimentary and geological material and the construction of plankton profiles south from New Zealand and Tasmania to the Antarctic ice.

Also back from the Antarctic is the Twentieth Soviet Antarctic Expedition. The programme of this expedition appears to have followed the usual pattern of meteorological and glaciological observations. Innovations included the automated processing of data obtained from upper atmosphere rockets—the first time, it is claimed, that this has been done under Antarctic conditions—and a new way of drilling glaciers using a borehole filled with a "non-freezing liquid". Drilling at the Vostok station was carried out to 450 m, and at the Novolazarevskii station (where no previous bores had been made) to 357 m—the total thickness of the icecap at this point. The TASS agency hailed this expedition as a notable achievement in international cooperation: it included medical workers and geodesists from East Germany; an American geologist wintered at the Molodezhaya station; and a Soviet glaciologist wintered at the US McMurdo base. A regular exchange of information was maintained with the British, Australian, US and Japanese Antarctic stations.

In contrast to these large-scale undertakings, a modest expedition of six skiers set off at the end of April from Wrangel Island to make their way over the ice to the drifting *Severnyi Polyus*-23 icefloe station. This expedition was somewhat misleadingly described by TASS as a "sports expedition", and although its close association with the newspaper of the young Communist movement at first suggests heroic adventure rather than serious research, this was not the case. One of the main aims of the expedition was the study of problems of psychological compatibility in a small team working under extreme conditions. The rations of the party were based on those of cosmonauts, and the results of the project may be intended for use in the study of the potential personality conflicts and stresses which may arise aboard a space-station or in long space flights. ☐

correspondence

More on Paradisia

Sir,—It is good to see you airing the Paradisia/Dominatia relationship in your columns (May 20, page 178). As a labourer in a different corner of the vineyard from Professor Brock, I find myself with another perspective. My concern is the training given to young Paradision Scientists (PhD students and post-doctoral fellows) when they visit Dominatia.

I think it is fair to assume that most Domination Scientists would like to help Paradisia develop and operate a self-supporting research effort—suited both to the intellectual and the material needs of the country—as soon as possible I sometimes wonder, however whether they set about things the right way. There may be States where the system to be aimed for closely resembles one to be found in Western Europe or the USA, but such places must form a small minority. The normal situation is one where at no time in his working life is today's young Paradisian academic likely to have the resources either of time (because of a heavy teaching commitment) or money to undertake research in a way comparable with his Domination counterparts. His high intelligence, diligence and determination cannot alter this fact, and it does no-one any good to attempt to ignore or disguise the situation. The ideal training for an individual of this kind is therefore different from that generally offered to natives of Dominatia. It needs, above all, to encourage independence and self-reliance (hard these days when research projects are often scarcely comprehended by a student before he starts to write his thesis). Experimental ingenuity with scarce resources and self-criticism allied with the optimism which attempts to cut a coat according to cloth (rather than weeping and complaining that good cloth is unavailable) are also essential.

In all of this Dominatia can help, but to be really useful involves more than treating students from Paradisia in the same way as their British counterparts. Doing so sometimes produces a fine scientist by Domination standards, but also someone who is discontented and unwilling to adapt to a different environment in the Paradisia to which he returns. Ideally a Domination supervisor will take the trouble to learn about Paradisia—its scientific research facilities, the peculiarly Paradisian problems which science might help to solve, even the government policies relevant to science and higher education. He needs to view the collaboration as part of the continuing development of his Paradisian colleague's life, as well as a contribution to his own research group's output. He should even, perhaps, begin to question whether his own view of the purpose of research, and what is worthwhile research, is the only tenable one. It may be that work which is not publishable in his own favourite journal can nonetheless provide important answers in a different context. In our joint efforts to help Paradisia, it is not only the Paradisians who need to learn.

Geoffrey R. Penzer

Cairo,
Egypt

PhD in evolution

Sir,—Your Editorial "PhD in evolution" (May 27) seems in its penultimate paragraph to reveal a general misconception about the purpose and nature of PhD research. It is assumed that PhD research should provide training for scientists going into industry. This, however, is not the case. The chief motive of a student going to a university science department to do research should be a desire to take part in the advancement of science. If he really wants to be trained for industry, he should go into industry, where he will receive a better training for that particular job than universities can provide. It is true that industry sometimes, not to say often, finds that PhD research has provided skills and attitudes which are useful to it, but this is not the primary purpose of PhD research.

Distorting university postgraduate research to provide a training explicitly and directly useful to industry will harm the progress of fundamental science and, conversely, a postgraduate research student entering a science department should expect to learn about fundamental research and the particular activities that entails. If he expects a course aimed at preparing him for industry he is embarking on university research with the wrong motive.

It may be said that, if PhD research is not providing a training for industry, there should not be many science research students. I think this view is correct; I believe university scientists should take on a research student only if he has a genuine desire to carry out original work in science and recognises that this may not help him to get a better job outside university.

These comments apply to PhD research in "pure" science departments; I believe the situation may be different in engineering and technological departments.

A. C. Rose-Innes

Department of Physics and
Electrical Engineering,
University of Manchester Institute
of Science and Technology,
Manchester, UK

The real AI

Sir,—Whilst bemoaning the antihumanistic potential of artificial intelligence (May 13, page 171), it is particularly inappropriate to make use of the long established and well recognised acronym for artificial insemination.

Yours faithfully,
Darrell H. Thorpe

London, UK

news and views

Discovery of superheavy elements

from Peter Hodgson

THE super-heavy elements 116, 124 and 126 have now been detected in the mineral monazite from Madagascar by a group of nuclear physicists from the University of Florida at Tallahassee, Oak Ridge National Laboratory and the University of California at Davis.

Over the past few decades a series of elements heavier than uranium have been discovered, mainly by successive addition of neutrons After plutonium and neptunium came elements such as fermium, einsteinium, mendelevium, nobelium and lawrencium. All are highly radioactive, and the heavier they are the shorter their half lives.

It has been known for a long time that the stability of nuclei is connected with their shell structure, those with magic numbers of neutrons or protons (corresponding to complete shells) being particularly stable. Lead 208 is especially stable, consisting of 126 neutrons and 82 protons, both magic numbers. The results suggested that the nuclei with even higher magic numbers of neutrons and protons might also be more stable than surrounding nuclei. It was considered very doubtful whether they would actually be stable, but it was thought possible that they would have half-lives long enough for them to be detected and their properties established.

The ensuing search for the offshore islands of nuclear stability or near-stability in regions of the periodic table beyond the peninsula of the heavy elements captured the imagination of nuclear physicists. Many different methods have been discussed, and among the most promising of these is the hope that the super-heavy elements (as they came to be called) could be produced in collisions between heavy ions at energies high enough to overcome their mutual electrostatic repulsion The difficulty is then to make them stick together, for there is so much energy in the system that they are very likely to fly apart again.

The possibility of such reactions was one of the strongest arguments put forward in support of the funding of heavy ion accelerators, and these are now operating or under construction in the USA, USSR, UK and other countries. At the same time there has been intense theoretical work devoted to the prediction of the properties of the super-heavy elements by extrapolation from the properties of the known nuclei. It is thus a great surprise that super-heavy nuclei have now been found to be stable and waiting to be found in a familiar mineral.

The presence of unusual elements in monazite was suggested by studies by Robert T. Gentry of the halos in mica caused by radioactive inclusions. Most of the halos could be identified as due to known radioactive nuclei, but a few could not. It proved possible to remove the inclusions from the mica and bombard them with 5.7 MeV protons from the Tallahassee Super FN Tandem Examination of the resulting X-rays by T. A. Cahill, R. C. Flocchini, N. R. Fletcher, H. C Kaufmann, L. R. Medsker and J. W. Nelson showed frequencies corresponding to the $L\beta_2$ transition in element 116 and the $L\alpha_1$ transitions in elements 124 and 126 These transitions were found only using the targets from inclusions showing the anomalous halos. These super-heavy elements are identified with 99% confidence, and in addition there is weak evidence for the elements 114, 125 and 127. The abundance of the super-heavy elements is about 10^{11} atoms per inclusion. Their chemistry is being studied by T. A Carlson and B. Fricke. The alpha and beta activity of these elements is being studied by J. D Fox and K. Kemper; the alpha particle energies are around 5 MeV

These results have stimulated intense theoretical activity in a group consisting of J. Berelacqua, D Stanley, P. J. Philpott, D. Robson, F Petrovich and M. Golin. Using the single-particle shell model, it is possible to obtain the energies of the single-particle states as eigen values of a one-body potential. The parameters of this potential can be fixed to give the energies of the known single-particle states in Pb, Sn and Ba. Using the known dependence of the one-body potential on A and on the nuclear asymmetry parameter $(N-Z)/A$ enables the energies of the single-particle states in heavier nuclei to be calculated. Calculations made some time ago indicated that $Z=114$ is likely to be the next nucleus of higher stability than its neighbours, so the experimental indications of $Z=116$ are somewhat puzzling. Calculations for $Z=126$ indicate that this also should be a stable nucleus, shown by the energy gaps between the filled and the next unfilled shells.

Calculations were also made for the nucleus $Z=164$ and this comes out to be particularly stable, with neutron and proton energy gaps of 2.9 and 2.4 MeV respectively. The energy of the alpha-particles from the decay of this nucleus was calculated to be 9.5 MeV, which corresponds very well with the unexplained 'giant halves' found in monazite. This nucleus has not yet been detected by the X-ray method, so this at present remains an unconfirmed possibility. If confirmed, it could account for the anomalous lead isotopic ratios found in monazite.

The alpha-particle half-lives were calculated for a range of super-heavy nuclei, and were found to be sufficiently large for them to be found naturally for elements 114, 124, 125, 126 and 127, but not for 116 and 128 So 116 is a puzzle in this respect as well. The element 164 was on the borderline of half-lives acceptable for stability.

There is certainly much more experimental and theoretical work to be done to confirm these results, to determine the structure of these new nuclei, and to account for their properties experimentally. This is a major breakthrough which will stimulate many new researchers in the months to come. ☐

Antiquity of tropical rain forests

from Peter D. Moore

It is often stated, and widely believed, that tropical rain forest is a vegetation type which has existed for a considerable length of geological time, some authors even suggest that no major evolutionary developments have taken place in such forests since Cretaceous times. In fact there is very little in the way of fossil evidence by which such claims can either be supported or refuted. Some new information, however, has been gleaned by Graham (*Evolution*, **29**, 723, 1976) as a result of a pollen analytical study of upper Miocene lignites from a lowland area of Coatzacoalcos, Veracruz, Mexico A hundred and ten pollen and spore taxa have been identified from these lignites and on the basis of these identifications some comparisons can be made with modern vegetation. True tropical rain forest taxa are poorly represented as are the arid community representatives now found in the area, but instead components of oak or pine–oak forest are frequent with possibly *Picea–Abies* forest at higher altitude. Reconstruction of past communities on the basis of pollen asssemblages is, however, fraught with problems, and as Graham gives little indication of the precise location of his sampling sites, the relative abundance of different taxa, or the methods used for determining palaeocommunities, it is difficult to appraise his conclusions critically.

Quaternary palynological work from other tropical areas such as the Galapagos (Colinvaux, *Nature*, **240**, 17; 1972), South America (van der Hammen, *J. Biogeog*, **1**, 3; 1974), and northern Australia (Kershaw, *Nature*, **251**, 222; 1974) certainly indicate that considerable changes in vegetation have occurred within the Pleistocene, though the reverse situation may obtain in certain areas, such as the Cuatro Ciénegas basin of Mexico (*Nature*, **247**, 129; 1974) It is not unreasonable to suppose, however, that changes were also proceeding within the Tertiary in some places The vegetation of many tropical forests may have undergone adjustments in its composition, presumably in response to climatic changes, and the concept of community migration, which is so popular with temperate palaeoecologists may also have relevance in the tropical context For example, the needle-leaved element in Graham's assemblage could represent an extension of the highland forests of Central America in response to lower temperatures.

Veblen (*Biol Conserv*. **9**, 141, 1976) describes such forests with a high proportion of coniferous trees from the highlands of Guatemala. *Abies* and *Pinus* are present in these forests, together with *Cupressus*, *Taxus*, *Taxodium* and *Podocarpus* and these may represent relict fragments of more extensive Tertiary coniferous forests Indeed, the abundance of species in the genus *Pinus* (29 spp) in the area has led Mirov (*The Genus Pinus*, Ronald Press, New York; 1967) to speculate that the highland areas of Guatemala and Mexico may have been a secondary centre of evolution for the genus.

The continued existence of these conifer-rich forests in Guatemala is of more than academic interest. Veblen points out that they represent an important genetic resource which is currently being eroded by exploitation for timber The land is subsequently used for agriculture, so that little regeneration is possible (Gomez–Pompa *et al.*, *Science*, **177**, 762; 1972). Severe genetic depletion in the forest communities is a real threat in these conditions since genetic races of various species will inevitably become extinct even where the species itself survives. Such a process must be regarded as an economic catastrophe in the long term, for these forests could supply the species so badly needed for the expansion of forestry in the tropics. So far only one of the highland Guatemalan conifers, *Cupressus lusitanica*, is used for this purpose; it has been planted in Africa very successfully and seems to have a wide climatic tolerance. Its timber production in Africa is said to be eleven times greater than that of species native to the plantation areas.

Fortunately these conifer forests of the uplands of Guatemala contain a rare and attractive bird, the Quetzal (*Pharomachrus mocinno*) which is the surest ticket any habitat can have for a rescue conservation effort of international proportions. The efforts in particular of the International Union for Conservation of Nature and the World Wildlife Fund led to the establishment in 1973 of a reserve of more than 400 ha of high altitude forest on the slopes of Volcano Atitlan in south-western Guatemala (La Basille, *Biol Conserv*. **5**, 60; 1973) According to Veblen, there is one other area where the Guatemalan forests seem to be fairly secure—the Department of Totonicapan, where the local Indian population has long exploited the forest in a balanced manner. To them the forest represents a source of income and it is therefore jealously guarded It is a sad reflection on modern society that the future of such potentially useful plant communities, with some claim to antiquity, now hangs in the balance. □

Red Sea: poles apart

from Peter J. Smith

Four years ago, Girdler and Darracott (*Comments Earth Sci. Geophys.*, **2**, 131; 1972) drew attention very briefly to the possibility that the Red Sea may have evolved in at least two separate stages of seafloor spreading. In addition, they raised, but made no attempt to answer, the question of whether or not the two phases of plate movement involved could be described by the same pole of rotation. In later presenting the evidence for a two-stage development of the Red Sea, Girdler and Styles (*Nature*, **247**, 7; 1974) made no reference to the secondary question of rotation poles, contenting themselves with describing the nature and dating of the Red Sea's geological history. But the omission did not go unnoticed; and Richardson and Harrison (*Earth Planet. Sci. Lett*, **30**, 135; 1976) have now rectified it with a conclusion not without geological implications.

According to Girdler and Styles, a depression may have existed in the Red Sea area as early as the Carboniferous and major rifting probably began during the lower to middle Eocene. The first phase of seafloor spreading then took place between 41 and 34 Myr ago, forming what is now the wider (southern) Red Sea (that is excluding the axial trough). The evidence for this spreading came chiefly from recognisable magnetic anomalies outside the axial trough and the timing was derived from best-matching the anomalies against different portions of the reversal time scale for different spread-

A hundred years ago

M W. DeFonvielle has had a spectroscope constructed with a graduated screen permitting the quantity of light admitted to be diminished in a known ratio. The moving force being regulated at will, the radiometer can be put in a state of rotation under the rays of the most scorching sun and record taken of the motion very easily. With such an apparatus it was shown by comparison with a standard oil-lamp burning forty-two grammes an hour, that on June 9, at 4 o'clock precisely, the radiating force of the sun was equal to fourteen lamps at a distance of twenty-five centimetres from the axis of the radiometer. The apparatus is tried daily at La Villette gas-works, and results of the comparisons will be tabulated and discussed.

From *Nature*, **14**, 181, June 22, 1876.

ing rates. The best spreading rate was 1.4 cm yr^{-1} over the 7 Myr.

This first spreading episode was followed by 30 Myr of quiescence during which great thicknesses (up to 5 km) of sediments, mainly evaporites, were deposited. Then 4–5 Myr ago a second phase of spreading began, splitting the older deeper oceanic crust with its heavy sediment load and creating the axial trough with new oceanic crust (and magnetic anomalies) rising to within 2–3 km of sealevel. This spreading phase continues today, giving the Red Sea axis a high heat flow and making it seismically active.

The Girdler–Styles model vindicated Davies and Tramontini (*Phil. Trans. R Soc*, **A267**, 181; 1970) to the extent that their seismic evidence had suggested that most of the Red Sea was underlain by oceanic crust while many workers were still maintaining that the undoubtedly oceanic axial trough gave way to Precambrian shield rocks. At the same time, however, it gave additional force to the question raised earlier by Girdler and Darracott In answering this question now, Richardson and Harrison accept the rotation pole at 36.5°N, 18.0°E for the total opening of the Red Sea, obtained by McKenzie *et al* (*Nature*, **226**, 1; 1970) by fitting together the coastlines. They point out, however, that given the validity of the Girdler–Styles model this pole will be the resultant of the first and second episodes of spreading. The overall NE–SW motion for Red Sea opening then may or may not be applicable to either of the spreading stages taken separately.

The lines joining congruent points of the opposing Red Sea coastlines are, of course, parts of small circles about the McKenzie *et al.* pole of rotation. According to Richardson and Harrison, however, the lines joining congruent points on the opposing sides of the axial trough are parts of small circles about a different pole at 15.2°S, 32.8°E. The axial trough thus seems to have been formed not by a NE–SW separation as for the Red Sea as a whole but by a roughly E–W separation Vectorial subtraction of the new axial trough pole from the overall pole of McKenzie *et al.* then gives a pole of rotation of 29.6°N, 20.6°E for the earlier phase of Red Sea spreading

One consequence of this calculation is that the Dead Sea rift, widely accepted as a transform fault, lies closer to a small circle of the new earlier pole than it does to a small circle of the McKenzie *et al.* pole; in others words, it seems to be a better behaved transform fault than previously supposed. Richardson and Harrison believe this to be "more than a fortuitous occurrence" and argue that the earlier phase of Red Sea spreading led to a chiefly left-lateral motion along the Dead Sea rift. But logic then dictates that the supposed change in direction of the second spreading phase should have imparted to the rift a large element of normal faulting. Whether such faulting can actually be observed is a matter of controversy, however. Most geologists familiar with the Red Sea area seem to dispute its existence, although a few claim to have seen its effects. It is a pity that Red Sea earthquake data are so poor that currently available fault plane solutions are incapable of resolving the question.

Genetic function of mitochondrial DNA

from D. Wilkie

An international conference on mitochondrial DNA was held at Riva dei Tessali under the auspices of the University of Bari from May 25 to 29, 1976. The organisers were C. Saccone and A. M. Kroon who will edit the proceedings to be published by North-Holland Publishing Co., Amsterdam. The publication date is July 30, 1976.

THIS was the latest in a series of Bari conferences on mitochondrial biogenesis which started in 1967. The predominant organism in this field continues to be the yeast *Saccharomyces cerevisiae* although contributions from those working with animal cells, trypanosomes, Paramecium and filamentous fungi are fast catching up and were well represented among the 35 papers presented. The main advantage in working with the yeast system is the availability of genetic analysis of mitochondrial mutants After the discovery had been made that mitochondrial drug resistance markers recombine in zygotes, the Slonimski group at Gif assiduously applied themselves to the genetic analysis of mutants, the most recent being the class known as *mit*$^{-}$ ("point" mutations in mtDNA) discovered by Tzagoloff and Needleman (New York) A further possible source of markers was suggested from evidence that mitochondrial factors can function in the regulation of nuclear genes (Evans and Wilkie, London). An up-to-date linkage map of the yeast genome was presented by Slonimski and Tzagoloff listing some 13 markers. If the *ts* mutant of Schweyen (Munich) and the RNA-coding segments are included (Rabinowitz, Chicago) reported 19 tRNA species identified so far, the map becomes quite extensive and may account for as much as 50% of the the total genes carried. The map obtained by genetic analysis corresponds well with results from deletion mapping in petite mutants using DNA–DNA hybridisation technique (Nagley and Linnane, Monash) and those obtained by following progressive loss of the genome at restrictive temperature in the *ts* mutant (Schweyen). In view of this progress, the mapping of genes in yeast mitochondria as an end in itself may no longer be justified and the emphasis should shift to the functional aspects of the markers. Since the pioneering studies of Roodyn and Work, it has been known that proteins are synthesized in mitochondria and yet a mitochondrial gene and its translation product remains to be identified in any detail. (Butow, Dallas) reported a correlation between mitochondrial mutants and variation in organelle proteins obtained by gel separation after labelling yeast cells and provided evidence of segregation and recombination of polypeptide species in crosses. (Grivell, Amsterdam) described attempts to identify mitochondrial gene products of yeast synthesised from mtDNA in a coupled transcription-translated system of *E coli* but results were complicated by interaction of translation products with proteins of the system.

Extensive use was made of bacterial restriction endonucleases in the analysis of mtDNA. This technique of obtaining precise fragment patterns is a powerful tool in the construction of physical linkage maps of mtDNA of all sources, coupled as it usually is with DNA–RNA hybridisation. Dawid (Baltimore) used the technique together with the electron microscope, in a comparative study of mtDNA of Drosophila, Xenopus and man (HeLa) and concluded that there was considerable base-sequence diversion between the species, indicating evolutionary diversification Saccone and Kroon (Bari and Groningen respectively) concentrated on the RNA from purified mitochondrial ribosomes of rat liver Hybridisation of RNA species of the 55S ribosomes with mtDNA fragments allowed construction of a map of rRNA-coding segments which mapped some distance apart Attardi (Caltech) working mainly with tRNAs of HeLa cell mitochondria, presented evidence that both light and heavy strands of mtDNA are transcribed into functional tRNAs. Symmetrical transcription of mtDNA necessitates a reappraisal upwards of our estimates of the coding capacity of mitochondrial genomes if this phenomenon is general. Preliminary results in other systems, for example of Rabinowitz with yeast, were in support of this. The original application of restriction enzyme analysis of mtDNA, namely to detect differences in fragment patterns between strains and mutants of yeast, was extended by Barnardi (Paris) in an elegant demonstration of recombination of mitochondrial genomes in yeast crosses: physical evidence of recombinational events was clearly seen in vegetative segregants with new fragment patterns compared with those of the parental strains. On purely statistical grounds, Bernardi drew the interesting conclusion that cleavage sites of restriction endonucleases may be GC-rich segments of the genome which are not themselves coding regions. Four papers were concerned with the complexity of kinetoplast DNA of trypanosomes. Endonuclease treatment and electron microscope studies indicated that the strange network of KDNA may be made up of aggregates and concentrates of several thousand mini-circles which individually have a contour length of 0.3μm. However, there may be more than one class of mini-circle as indicated by sequence heterogeneity, more than one sedimentation peak and little renaturation of mini-circle preparations.

The mtDNA of *Paramecium aurelia* is circular but the circles may vary in size (Cummings, Colorado) Borst (Amsterdam) could provide no evidence that the mtDNA of Tetrahymena is other than linear and showed that replication intermediates are also linear, so this organism remains the odd one out in this respect. Systems for transference of mitochondrial type from one cell to another in Paramecium (Tait, Edinburgh) and in mammalian tissue culture (Eisenstadt, New Haven) were described as well as a system for the expression of mouse mtDNA in prokaryotic and eukaryotic cells (Clayton, Stanford).

A million memories?

from Andrew Holmes-Siedle

SOLID-STATE physicists have been refining their views on how far the miniaturisation of semiconductor circuitry can go (Wallmark, *Institute Phys. Conf. Ser.* No. 25, 133; 1975 and Keyes, *Proc Inst. Elect. Engng*, **63**, 740; 1975) and it is thus worth seeing how near the integrated-circuit industry is coming to the physical limits which have been defined. Such is the urgency of the competitive rush to cram circuits on to silicon chips in larger and larger numbers and to operate them at high rates that it would seem that, if the rate of size reduction maintained its pace, designers would soon face fundamental barriers such as the speed of light, the onset of avalanche breakdown in semi-conductors and the randomness of impurity distribution. The field of semiconductor memories is at the centre of this competition.

The electronics journals are pulsating with advice and controversy over which of the dozens of designs of silicon memory chip are the best to use. Chips measuring 0.3×3×3 mm and carrying 4,096 bits of information can now be made cheaply (say for £5) and there is speculation that next year the semiconductor industry may be able to mass produce a chip containing 16,384 bits with little increase in chip size. Both devices are in demand for installation into new minicomputers and, in addition, all the logical operations will soon be performed on a single "central processor" chip (the "microcomputer"). Also, advanced technology laboratories are already hard at work on the 65,536-bit (2^{16}-bit) memory chip, which might be produced by 1979.

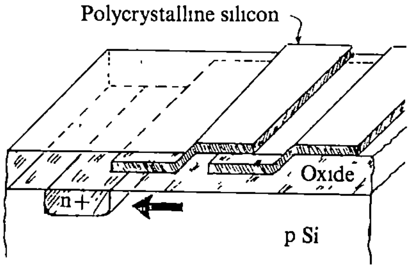


Fig. 1 Two level electrodes.

I wish to show that in this "memory chip" field, we may get all we need in the way of storing information in a small space without meeting limitations due to physical laws. I submit that we probably store about a million (2^{20}) bits on a single chip of silicon only just over half an inch on a side (say 15×15 mm) without great efforts and that we will then probably stop trying to go further because this capacity is adequate for most purposes where miniature size and low weight are important, and the cost of even greater density may be prohibitive.

As I have discussed before (*Nature*, **257**, 739; 1975), several physical constraints come in when the width of an individual line in the circuit pattern becomes less than about 2 μm (for example if a particular diffused junction region must have dimensions less than 2×2 μm). Several tricks in metal-oxide-semiconductor (MOS) device technology, however, have been devised which make it possible to pack in more functions per square micrometre without reducing the individual line widths. First, the use of multilayer conductor geometry enables two electrodes to overlap (Fig. 1); second, the surface of the silicon is now being etched into grooves (Fig. 2), which leads to the first example of a vertical MOS device, and third, the number of electrodes needed to perform a function is being reduced by the invention of new device principles. For example, using the memory principle shown in Fig. 3, the charge-coupled random access memory (CC-RAM) it seems possible for information to be stored in a cell of 100 μm² (10×10 μm) or smaller. The cell structure is so simple that the individual line widths used need not be less than 3 μm. As 1 cm² is 10^8 μm², it is easy to get a million bits on to an area smaller than a thumbnail. In fact, allowing extra 'real estate' for leads, amplifiers and wiring contacts, the million-bit chip would probably be 15×15 mm or 0.6×0.6 inch. If we consider about a thousand of these chips mounted in individual ceramic packages and then on circuit boards, and stacked in the corner of a room, we could easily imagine a capacity of a few billion bits of memory in a small cabinet. Would we want to go further in capacity in this form? Probably not; the *Encyclopaedia Britannica* only contains 40 million words and any larger storage requirement, say the keeping of archives or the storing of video information (a billion bits is only a few video frames), can quite often be handled by the use of disks or tapes.

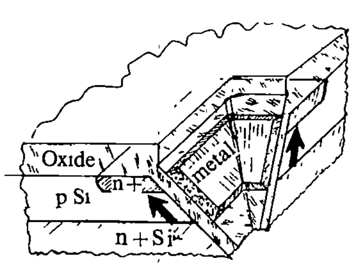


Fig. 2 Use of depth in Si.

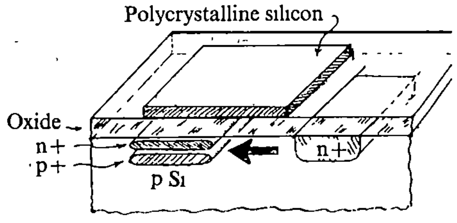
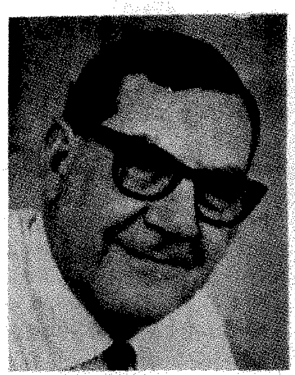


Fig. 3 Use of new electrodeless effects.

This is only to say then that, in the semiconductor field, the basic device phenomena are available to take us to the small "mass memory" density needed in small, portable computers. There will probably be at least half a decade of technological effort before the million-bit density is achieved at reasonable cost. The technology which will challenge magnetic tapes will be of an entirely different character and will probably involve light holography. □

CSIRO: fifty years of research

CSIRO, the Commonwealth Scientific and Industrial Research Organisation of Australia, celebrates its fiftieth birthday this year. We present in this special supplement a cross-section of the activities of an organisation employing over 2,000 professional scientists. Sir Robert Price, Chairman since 1970, introduces the review.



Looking to the future

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Although research in CSIRO will be determined by the significant advances in science as a whole, much emphasis will still be on the use of resources, the environment and consumer-oriented research. This is discussed against a background of increasing interaction between science and politics.

ALTHOUGH any attempt to assess the future role of an organisation such as CSIRO can only be highly speculative, it is valuable to try to do so even if nothing more is achieved than the identification of existing deficiencies. But whatever the outcome, it is necessary to devote a little time to considering some of the events that have contributed to the present situation in Australia with regard to science and technology and to the role of CSIRO.

A suitable starting point is the establishment, in 1926, of the Council for Scientific and Industrial Research (CSIR). Among its terms of reference was a major requirement that it initiate and carry out "scientific researches in connection with, or for the promotion of, primary or secondary industries". The Council was given considerable freedom to achieve its aims, and one of its first decisions—a crucial one—was that it should carry out its own research, with its own staff, in its own laboratories. To do this, it set up a number of research divisions and to this day the division remains the basic organisational unit of research in CSIRO.

Initially the emphasis of the Council's work was almost entirely on primary industry, and a succession of useful discoveries helped the new organisation gain acceptance by that industry and by the community in general. This encouraged the Government, in 1936, to provide resources to extend the activities of CSIR into areas of research for secondary industry. This proved to be a wise decision because of the part played by the newly established divisions in the wartime development of the Australian manufacturing industry.

Sir Frederick White deals in the next article with some aspects of those early years and has outlined the background to the transformation in 1949 of the Council (CSIR) into the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Referring to the early years of CSIRO, Sir Frederick writes of that period as one in which money was easy to obtain. It was a period, spanning the 1950s and most of the 1960s, of comparative prosperity and stability for Australia, a period which saw the setting up of the Murray Committee on Australian Universities and the implementation of that Committee's recommendations. These met the pressing need for additional support for existing universities and led to the establishment of new universities and to a considerable expansion in university research. These important steps, which had the full support of the Executive of CSIRO—the Chairman, Sir Ian Clunies-Ross, was a member of the Murray Committee—led to a much improved balance in the national research effort between university and government laboratories and, incidentally, reduced a measure of undesirable friction which existed between some university scientific staff and CSIRO.

There was, and still is, however, a serious imbalance as far as industrial research is concerned, a situation which was bettered to a limited extent in that same period by the introduction of industrial research and development grants by the government, a step which was also actively supported by CSIRO. At present, then, the inadequate level of research activity in manufacturing industry is in marked contrast to the substantial proportion of the country's total research effort represented by government research agencies of which the largest is CSIRO.

By the late 1960s CSIRO had grown substantially, setting up new divisions and expanding and changing the direction of existing divisions, and in spite of very limited growth during the 1970s the total staff now exceeds 7,000, one-third of whom are professional scientists. As we moved into the 1970s, however, it became increasingly evident that CSIRO

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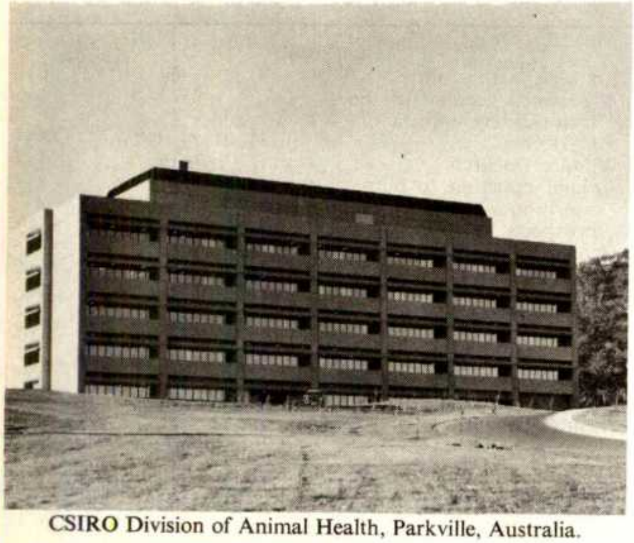
—indeed, Australian science and technology as a whole—would have to adapt to a rapidly changing economic, social, and political climate. Similar pressures had already been encountered by governmental research organisations in other countries. In Britain, in Canada, and in the USA, for example, government-funded research agencies have, in the past decade, had to contend with a variety of problems, partly financial but significantly concerned with the relationships between science, perceived national needs, and often unperceived social deficiencies.

Nevertheless, there is no doubt that the most significant factor affecting Australian science and technology, and certainly CSIRO, in recent years, has been the growing involvement of science with politics. Politicians are becoming increasingly aware of science, and scientists can no longer count on the uncritical support of governments. A very important factor in bringing about this change was the establishment of a Ministry for Science—initially a Ministry for Education and Science.

Although this increasing interaction between CSIRO and government, and the growing need to justify policies and programmes, has not affected the integrity and quality of research in CSIRO, it can in some cases, and properly so, influence the choice of a particular research programme or the relative levels of support in certain areas.

A criticism of CSIRO which one encounters from time to time is that it is too large. Those who offer this criticism rarely state what they mean by "too large"—whether for effective management or because the Organisation receives what is thought to be too large a slice of the financial cake provided for science and technology by government. Yet in the size of CSIRO lies one of its great strengths. The sense in which I use the word 'size' relates not just to numbers of people employed or to budgets but to the wide diversity of scientific and technological activities embraced by CSIRO's programmes.

This aggregation of scientific skills and facilities is not only exceedingly valuable now but will become even more so in the future, for two reasons. First, because of the growing importance of collaborative multi-disciplinary research which is most easily achieved within one organisation. Collaboration with other bodies, both nationally and internationally, is of course still important and will be even more so in the future. Such collaboration, however, tends to operate on a more formal basis and cannot always be entered into quite so readily or operated so efficiently. Second, because of the broad base of activities there is almost always a nucleus in one or more divisions which can be developed and built on to initiate research on new problems arising from changing community needs or changing economic conditions. These two reasons constitute a very strong case for the retention of a broadly based governmental research organisation such as CSIRO, although there will always be a need for other more specialised bodies.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Against that background let me try to assess the role that CSIRO may have as government and community demands on scientists change, as change they must.

I repeat that the broad base on which CSIRO operates will make it easier to cope with changing needs with minimal delay or upheaval. These changing needs will not create problems different in kind from those which CSIRO has already faced. It is unlikely, however, that CSIRO will, in the foreseeable future, experience growth rates comparable with those of the 1950s and 1960s. Consequently it will need to develop the ability to redeploy resources in response to changing priorities to a degree that has not been necessary in the past.

But it is not sufficient to consider CSIRO in isolation. It is only one component in the total structure of scientific research in the Australian community and it must maintain and improve relationships with other components. A criticism of CSIRO which I think has some validity is that during the period of expansion to which I have referred the Executive was unduly inward looking, perhaps understandably in the circumstances. The changes which have taken place in the 1970s have forced on us acceptance of the need for more effective external relations with various official bodies and community groups—in particular, with industry, with Departments of State, with universities and with other bodies either involved in research or having research requirements, including the Australian Science and Technology Council. At present, CSIRO supports research in universities on only a very small scale and only infrequently gives research contracts to industry. There are good reasons why the letting of research contracts by CSIRO to universities, industry and other institutions, should be expanded considerably, and I envisage that this will occur in the future.

Although the role of CSIRO will continue to be one which rests essentially on the application of the natural sciences, there is no doubt that the problems of the future require an increasing input from social scientists. The Organisation has already recognised this by recruiting small numbers of social scientists into Divisions such as Land Use Research and Building Research. I envisage a considerable increase in recruitment of social scientists, their wider distribution throughout CSIRO, and a wider spread of socially oriented programmes across divisions.

I do not propose to make predictions in this article about those fields of research on which CSIRO is likely to concentrate in the future, although it is clear that there will be emphasis for a long time to come on such obvious areas as resources, renewable and non-renewable, and on environmental and consumer-oriented research. The directions of CSIRO's future research programmes will be determined by the significant areas of advancement of science as a whole, by the requirements of the country as seen both by the scientists and the politicians as expressions of community needs, and by the practicalities of the human resources and material facilities available.

In the final analysis, the Executive is responsible to Parliament for ensuring that the Organisation's resources are used efficiently and, in terms of the needs of the Australian community, effectively. The Executive's ability to achieve this in the future will hinge on CSIRO remaining an elitist organisation recruiting the best research scientists it can on a worldwide basis, on providing the best possible facilities consistent with the resources available, and on maintaining within the Organisation an environment that is conducive to creative and productive research.

A personal account of the historical development of CSIRO

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The origins of CSIRO can be traced back to the foundation in 1916 of the DSIR, under the pressure of scientists, for a greater use of science in industry. After some initial uncertainty as to how to run such an organisation, CSIR was set up in 1926. Early research was devoted to agriculture, but in the late 1930s CSIR redirected its thrust towards industrial research. Under a McCarthy-like scare in 1949, the CSIR's name was changed to the CSIRO and since then it has continued to produce important work on a wide variety of topics.

AUSTRALIANS were first stimulated to form a national research institution by the news of the proposal in 1915 in Britain, during the First World War, for greater use of science for national efficiency and progress. A deputation representing learned societies, led by Sir William Crook's, President of the Royal Society put this proposal to the British Government; later in that year A. J. Pease, President of the Board of Education, announced to the Commons a scheme for an advisory committee for scientific research. Arthur Henderson, who became the President of the Board of Education when the government changed, presented as a White Paper to both Houses of Parliament *A Scheme for the Organisation and Development of Scientific and Industrial Research.* By Order-in-Council of July 28, 1915, a Committee of the Privy Council for Scientific and Industrial Research and an Advisory Council were formally established. It was from these beginnings that there emerged the Department of Scientific and Industrial Research (DSIR) whose excellent activities many scientists in Britain and overseas remember. It set a pattern of Government sponsorship of research in science; DSIR generously assisted younger and similar institutions in what we used to call the British Empire.

Because of the initiative of prominent academic leaders, one of whom was David Rivett's predecessor as Professor of Chemistry in Melbourne, David Orme Masson FRS, an Advisory Council for Science and Industry was set up in 1916 by the Australian Government of Mr W. M. (Billy) Hughes. Although it had wide terms of reference it did not succeeed in establishing continuing national research. Its one success, however, the support of research on local hardwood timbers led, eventually, to the development of the pulp and paper industry.

By 1920 it was apparent that the Advisory Council was not an effective arrangement. The responsible members of the Council, led by Orme Masson, eventually, though with difficulty, persuaded the Prime Minister to pass a Bill creating the Institute of Science and Industry (1920). The powers and functions specified in the Act are similar to those written into the Science and Industry Research Act which created the Council for Scientific and Industrial Research (CSIR) in 1926. A full-time Director alone had complete authority for the management of the Institute. The comprehensive plan for research recommended by the Director, Sir George Knibbs, in 1921, could not be implemented for lack of finance and again this attempt to form a permanent national institution failed.

When the government changed in 1923, Mr S. M. Bruce, later Lord Bruce, became the new Prime Minister. Bruce and his Ministers accepted the idea that Australia's development depended greatly on her ability to apply scientific methods to industry. The Prime Minister acted with speed and determination to alter and to improve the arrangements for this objective. In 1925 he personally presided over an authoritative conference of senior academic, industrial and political leaders from all States of the Commonwealth to discuss the reorganisation required. Sir Frank Heath, Secretary of the Department of Scientific and Industrial Research of Britain was invited to Australia and reported his views to the Government in January 1926.

Having decided that the Institute of Science and Industry Act (1920) must be amended, the Prime Minister invited George Julius, David Rivett and an engineer named Newbiggin to assist in drafting the Bill for an Act to found CSIR.

The Science and Industry Research Act was passed by the Parliament of the Commonwealth and given Royal Assent on June 21 and the Council had its first formal meeting on June 22–25, 1926.

When I went to Melbourne in 1945, just before my appointment to the Executive Committee of CSIR its members were Julius, Rivett and Richardson; Newbiggin had died in 1927 and Richardson had been appointed in his place. The determination and wisdom of these three carried this venture forward successfully for twenty years.

George Julius had distinguished himself as an engineer and at that time had a flourishing consulting practice in Sydney. He was an attractive and vigorous talker on all occasions. When chosen by the Prime Minister in 1926 to be the part-time Chairman, he was already known for his activities in the Institution of Engineers of Australia, through which he and others founded the Standards Association of Australia. He had a wide interest in many aspects of the economic life of his country.

Left to right: George Julius, Sir David Rivett, Dr A. E. V. Richardson.

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*Sir Frederick White was Chairman of CSIRO from 1959 to 1970.

David Rivett had been persuaded to give up his Chair and his personal research in chemistry at Melbourne University to become the first full-time Chief Executive Officer of the new institution. His connection with the University of Oxford, where he went as a Rhodes Scholar from Melbourne, his friendship with Fellows of the Royal Society of London and with many eminent scientific men in England and Europe brought the CSIR into the environment of European science in the days when Australian scientists were few in number and lived at remote distances from that environment. David Rivett was a serious, kindly man who delighted in discussing with his scientific colleagues their work in the CSIR laboratories.

Richardson, always known by his initials A.E.V., joined the Executive Committee after being successively Superintendent of Agriculture in Victoria, Dean of Agriculture at Melbourne University, and the founding Director of the Waite Agricultural Research Institute in Adelaide. His profound knowledge of agriculture had a marked influence on the early programme of CSIR. Although, when I knew him, it was difficult for him to intervene in the flow of rhetoric of Julius, unless asked a specific question by Rivett, his deliberate statements were full of precise data and quite convincing.

CSIR consisted of the Executive Committee of three nominated by the Minister and appointed by the Governor-General, the Chairmen of the State Committees, and "such other members as the Council, with the consent of the Minister, co-opts by reason of their scientific knowledge."

The inclusion of the State Committees reflected the desire for a truly national activity, since acting through their Chairmen, the State Committees could refer to the Council problems considered important in their area. The co-option of additional members enabled the Council to provide itself with a wide variety of industrial, academic, agricultural or other advice.

It was not in the nature of Julius, Rivett or Richardson to do other than take the initiative; they did this with considerable success while deferring, as they formally had to do, to the Council.

Of prime importance to success was the fact that the Act placed in the hands of the Council the full authority to make such appointments to the staff on such terms and conditions as it felt necessary for the carrying out of the work to be undertaken subject only to the Minister's approval. The CSIR did not have to conform to the usual employment arrangements of the Commonwealth Public Service; the Executive Committee and the Council had a remarkable degree of freedom of choice of individuals and freedom to establish the conditions of employment that were thought most appropriate.

In the very early months two ideas were rejected. These were that the work of the new institution could be carried out under the guidance of specialist committees, and that the work itself could be undertaken in existing institutions, either State Departments of Agriculture or the universities. In the earliest discussions Julius took the initiative in rejecting the idea of committee control, holding that investigations undertaken in such a way would be protracted, that the committees would have difficulty in meeting, and would not be able to give the guidance and expedition to the activities that he desired. The Council agreed to appoint 'experts' to take control of related groups of investigations and passed a resolution "that in the opinion of this Council the problems awaiting solution are so vast and difficult that it is essential that men of outstanding quality should be secured to devote the whole of their time to efforts at solution".

Julius probably had some diplomatic difficulty in sidestepping the idea that the laboratories of other institutions could provide the venue for CSIR's investigations. Rivett, Richardson and the Council supported him and CSIR avoided having to give way to pressures in this direction. Indeed one of the earliest decisions made was that the Council should establish its own laboratories. With regard to universities, the Council decided that it would be quite unsatisfactory to leave the important problems of the CSIR to be dealt with as the spare-time activities of the staffs of the universities.

Although a quarter of a century had passed since, by agreement with the States, the Australian Commonweatlh was founded, the question of State–Commonwealth collaboration was still difficult particularly in specific areas. The founding of CSIR was not altogether welcomed by the State Departments of Agriculture which had been in existence since the very early days and which felt that they had a responsibility, which they were discharging effectively, to assist in the development and promotion of agriculture. This was important, particularly at that time, since the economic life of Australia was largely dependent on the agricultural and pastoral industries. Even Julius, the enthusiastic engineer, was one of the first to promote the idea that CSIR's early programme must be related mainly to the primary industries of the country. It was, therefore, essential that some agreement be reached with the States in the field of agricultural science.

The resolutions passed at a meeting between representatives of the Council and the State Departments of Agriculture, held in 1927, clearly outlined the basis for the division of research functions and did this in a way which set a tone for CSIR's future research. The conference confirmed the number and magnitude of the problems confronting the agricultural and livestock industries, it welcomed Commonwealth participation in research, and stressed that the field was almost unlimited in scope and that there were many problems, regional or national in range, and fundamental in character, which required concentration of effort and highly specialised research for their solution. The conference agreed that these problems were specially suited for investigation by the Commonwealth. In other words, the meeting handed over to CSIR, and I should imagine very much to Rivett's satisfaction, the more difficult long term problems that required scientific research in depth.

And so the Council began its task and must have done so with optimism for the future. In 1926, when the Act was passed, a sum of £250,000 was appropriated and placed in a Science and Investigation Trust Account for the purposes of the Council. In 1928 a further £250,000 was appropriated and placed in this account.

It is difficult today, so many years later, to envisage the Australia of that time. The main economic activities were rural, wheat and other crops, sheep, wool and beef cattle.

The first laboratories to be established were for the study of plant problems and entomology, in Canberra, animal health and nutrition in Melbourne and Adelaide and soils in Adelaide in association with the Waite Agricultural Research Institute. Studies of forest products grew out of early work in Perth but the main effort developed in Melbourne. A laboratory for fisheries investigation was established a few years later. The export of beef and lamb to the British market presented problems of cold storage which were to be studied, together with other problems of the food sciences, in laboratories in Sydney and Brisbane. The Australian Radio Research Board inspired by a similar arrangement in Britain, was set up and with a grant from CSIR and the Post Office began to support research in the universities.

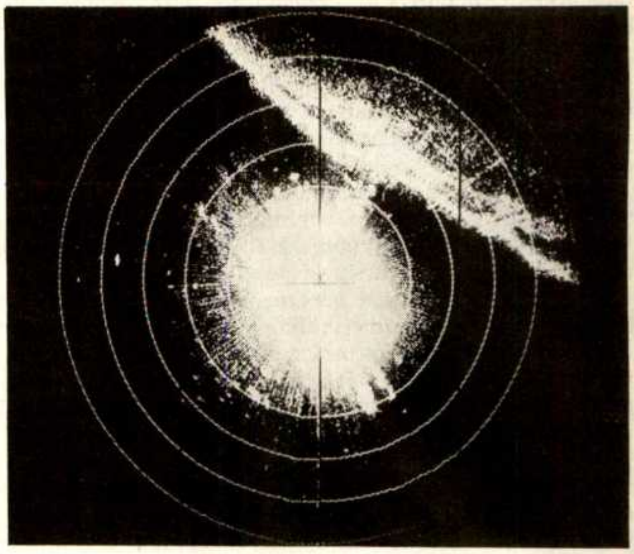
After these initial successes, the confidence of the Council was severely challenged when in 1930 the financial difficulties of the Commonwealth began to have an influence. The interest of the government was still strong but so serious was the financial position that the Trust Fund, already depleted, could not be adequately recouped. Survival depended on garnts made to the Council by the

Empire Marketing Board, the Australian Pastoralists Research Trust and particularly the Rural Credits Development Fund of the Commonwealth Bank. To the relief of the Executive Committee, the Bank, in response to special pleading by Bruce in 1932–33, found £21,500 to make up the already severely reduced annual budget of £112,000. The Rural Credits Development Fund began making grants to CSIR in 1930 and continued to do so until 1939.

Having successfully ridden out the trials and tribulations of the depression years, the Council was prepared for the next great leap forward. The economic scene was changing with rural production being augmented by the production of the manufacturing industries. At the end of the First World War, the motor car was a curiosity but by the end of the 1930s, motor cars were common on the roads of Australia. As the depression years passed, a more prosperous community could take advantage of the general improvement in the quality and comfort of motor cars. Australian industry built the car bodies into which imported engines from the USA or Canada were fitted. This industry stimulated the manufacture of sheet steel, timber and leather products, paints, varnishes, as well as the manufacture of springs, bumper bars, shock absorbers, and so on. In the electrical field the use of what are today regarded as common appliances in the home was becoming more widespread. Firms such as Philips, Thompson Houston and the Australian General Electric, began manufacturing in Australia, or importing parts to be assembled here. Generators, transformers, telephones, and later refrigerators and domestic appliances generally were being made. Public broadcasting by radio began in 1920 and the manufacture of radio receivers began using imported parts; later the components were made by local industry. Likewise government expenditure on development was having its effect. There was a growing demand by public authorities for the construction of roads, irrigation dams and channels. The use of reinforced concrete was revolutionising building construction. Broken Hill Pty., and its associated companies, had begun steel production in Newcastle in 1915. By 1930, 25% of the output went as coiled rod to the wire industry for barbed wire, nails, fencing wire and netting. Much of the steel went into galvanised iron and a great deal of it was used to manufacture rails and other materials for the developing Commonwealth and State Railways. The scene was changing rapidly and very much in the direction of the interests of George Julius.

The Executive had been under pressure for some time to extend the activities of CSIR towards the interests of secondary industry. Julius, in 1936, saw in the government's request for research connected with the manufacture of engines for aircraft and cars the opportunity to advocate a wide ranging enquiry as to what the Council should do in the whole field of secondary industry research. The government appointed Julius chairman of a representative committee of members drawn from the engineering profession, industry, CSIR and the academic world to make a complete review of what was required. The recommendations of this Secondary Industry Testing and Research Committee, when adopted by the Government, transformed the activities of the Council.

The National Standards Laboratory was founded, and the beginnings of an Information Service approved. The setting up of the Aeronautical Research Laboratory and the Department of Aeronautics in the University of Sydney followed the special advice of H. E. Wimperis, Director of Scientific Research at the Air Ministry in the UK. At the same time the decision was taken to found the Division of Industrial Chemistry. When the war broke out in 1939 all these and some other activities connected with secondary industry were in the planning stage. By 1945, when the war ended, no less than 350 professional staff were employed in research related to secondary industry, a number greater than had been employed in the whole of the activities of CSIR before the outbreak of the war.



Radar location of locusts.

Julius was in his element with the Division of Aeronautics and its major constructional undertakings for windtunnels and the like. This was a very successful venture but fell on hard times later when it had to be transferred from CSIR to the Department of Supply because of the rejection of secret work in CSIR. During the war, it was a flourishing institution, for the interest of the Royal Australian Air Force predominated. Rivett was happy with the development of the Division of Industrial Chemistry although there are indications that he had doubts about the rapidity of secondary industry research growth.

Professor J. P. V. Madsen, Professor of Electrical Engineering in the University of Sydney, had a special role in the founding of the National Standards Laboratory (NSL, now called the National Measurement Laboratory). Shortly after CSIRO began he made an approach to the Executive Committee advocating the need for primary metrological standards of physical quantities to aid industrial development. Although he chaired several committees his efforts were unsuccessful until supported by the government following recommendations by the Secondary Industry Committee. Madsen was given special authority by the Council to acquire a site to supervise the construction of a building and the allocation of the work of the different sections as they were formed.

The senior officers appointed to the National Standards Laboratory were in training in England at the National Physical Laboratory when the war broke out. They returned in time for the NSL to take part, with the Munitions Supply Laboratories, in providing a comprehensive measurement service to the munitions manufacturing industry. When the war ended NSL was able to take up its major role as the national laboratory for research on metrological standards and the measurement of physical quantities similar to the NPL in Britain and the National Bureau of Standards (NBS) in the USA.

Among the many men, members of the Council, industrialists or academics who had a role in the activities of CSIR, and later CSIRO, Madsen was outstanding among them. He graduated in engineering in Sydney, worked with W. H. Bragg in physics research in Adelaide and was appointed the first professor of electrical engineering in Australia: he devoted his life to the promotion of research in physics and engineering in the universities and in CSIR.

In 1939 the British Government invited Australia to send a representative to England to learn of the secret development of radar. Acting on Madsen's advice, the Government and the Executive Committee selected Dr D. F. Martyn, an officer of the Radio Research Board for this mission. When Martyn returned, the Government agreed to set up a special radar laboratory in Sydney to play the part in Australia, although in a smaller way, played by Telecommunications Research Establishment (TRE) and other similar institutions in England. The Radiophysics Laboratory was founded and the staff grew during the war to 65 professional scientists.

It is questionable whether the activities recommended by the Secondary Industry Testing and Research Committee would have been accepted, let alone made such vigorous progress, if the war had not occurred. Because CSIR agreed to undertake important wartime work, money was readily available and growth was rapid.

When the war ended CSIR found itself with four major and several smaller institutions with programmes that had then to be changed to a peacetime civil basis.

Sir George Julius retired as part-time Chairman in 1945 and died in June of the next year, aged 73. David Rivett agreed to become the new part-time Chairman and Dr Ian Clunies-Ross and I, both appointed to the Executive Committee sometime earlier, continued with him as the new administration.

In retrospect it is clear how fortunate we were that David Rivett was the Chairman of CSIR when the political storm broke over us in 1948. The Liberal-Country Party, in opposition, attacked the Labour Government with accusations that the universities and CSIR were employing disloyal scientists with communist sympathies. It was alleged that important information from the USA was, as a result, being denied to Australia. Rivett was attacked personally when he advocated the return of CSIR to civil science and the setting up of separate defence research laboratories. The Opposition wanted CSIR brought more directly under the control of the Government. As is usual in these controversies, a great number of statements were made which were erroneous, irrelevant and of little real significance. The Prime Minister, then Mr Ben Chifley, and the Minister in charge of CSIR, Mr. John Dedman, took this matter very seriously. After due enquiry, the Cabinet decided that the Act under which CSIR worked had to be changed particularly to relieve the Minister of all personal responsibility for the appointment of staff.

It was Rivett's prestige, and the appreciation of Chifley and Dedman of what he and his colleagues had done for Australia that led the Government to insist only on essential changes in the Science and Industry Research Act. Indeed the new Act of 1949 placed greater responsibility on the 'Executive', of five members. The Executive would in future be responsible, with a few minor exceptions, for all appointments to the staff.

The new Advisory Council in this Act retained the same form as the old Council but had none of its administrative powers. With no governing Council the name had to be changed to the "Commonwealth Scientific and Industrial Research Organisation" (CSIRO). The "powers and functions" of CSIR were transferred unchanged to CSIRO and the scientific programme continued as before.

Rivett was, naturally, greatly distressed that the Government did not turn to the Council for advice. In retrospect, it is clear that the Government had to insist on certain changes to protect itself and the responsible Minister from false accusations. In spite of his disappointment and distress, Rivett, now nearly 64 years of age, who had reached the end of his formal period of appointment, consented to become the part-time Chairman of the new Advisory Council. He occupied this post for about a year and then became a part-time member of the Advisory Council for the following six years.

The proclamation of the new Act introduced a completely new regime. Ian Clunies-Ross was appointed the Chairman of the Executive, I was the Chief Executive Officer, and Dr S. H. Bastow, a physical chemist, was appointed as our third full-time scientific colleague. The two part-time members were Mr H. J. Goodes, an officer of the Treasury and Mr Don Mountjoy of Western Australia. Mr A. B. Richie, a grazier from the Western District of Victoria, replaced Mr Mountjoy in 1950.

The new administration entered into its responsibilities in a period of post-war euphoria. Money was easy to obtain and, indeed, the increase in our annual appropriation from the Commonwealth Government was quite large so growth, therefore, followed suit. We were able to introduce not only increases in the existing activity but some interesting innovations.

Pasture research, so successful in the south, was extended to the tropics using domesticated wild species of grasses and legumes from overseas. Animals more suited to the tropics than British breeds resulted from genetic studies of the beef-producing breeds. The physical and biological features of Northern Australia are now better known following extensive land-research surveys using novel methods. The unexpected widespread myxomatosis epizootic not only reduced the enormous rabbit plague to reasonable levels but was the opportunity for studies of the evolution of a virus in nature.

Money made available by the industry permitted a major expansion of research on the healthy sheep as a wool- and meat-producing animal. Wool textile research evolved new spinning methods and many novel treatments of wool textiles that have helped the International Wool Secretariat to promote wool in competition with synthetics.

Knowledge of the meteorology of the Australian sector of the Southern Hemisphere has been greatly enhanced since the beginning of early researches in meteorological physics. The National Measurement Laboratory is now a respected partner of the British NPL and the US NBS. Many researches of scientific and practical merit have over the post-war years emerged from what was originally the Division of Industrial Chemistry, now subdivided into several major independent laboratories; probably the most widely known is the development of the technique of atomic absorption spectroscopy.

The Radiophysics Laboratory, when work on radar ended, planned several peacetime researches. The curiosity of the late Dr J. L. Pawsey about extra-terrestrial radio noise took a major group of his colleagues into the new science of radioastronomy. By 1952, when the URSI met in Australia, no laboratory in the world could claim to have played a greater part in this new science.

The conquering of the pleuro-pneumonia epidemic in cattle, advances in cloud and rain physics and in the food sciences together with a major attack on the science of Australia's mineral resources are only a few examples of diversity of the research programme and its growth since 1945.

I have written perhaps too much of the Act, of the Executive and Council and said less than is adequate about the leaders of research whose imaginative concepts and scientific abilities made possible the achievements of CSIR–CSIRO. This is clearly inevitable in an account of how this institution started, survived the rigours of the depression and the war and escaped fatal change as a result of political expediency.

The early decision of the Council to appoint "men of outstanding quality" has been amply justified; it would be invidious and even tedious here to name and describe the work of the many men and women who have led the advance in pure and applied research. The record of their recognition by Australian and overseas learned societies and universities will speak for them.

Wildlife research

H. J. Frith

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The first work in CSIRO on wildlife was on the control of the rabbit, and this work still continues. But many other animals regarded as pests have also been investigated, from the Tasmanian waterhen to the kangaroo. Not all research has been devoted to pests, however, and recently the division has been pursuing an increasing amount of research on native fauna for its own sake.

WILDLIFE research began in the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in 1949. Before that there had been suggestions that the study of Australia's native fauna would be a desirable role for the Organisation but, in the economic atmosphere of those immediately post-war years and in the face of the enormous populations of rabbits that had developed due to the shortage of manpower and materials for control, the primary aim of CSIRO in forming the Wildlife Survey Section (later Division of Wildlife Research) was the study of the rabbit. Even so the founder, the late Dr F. N. Ratcliffe, saw beyond the immediate objective and anticipated that, ultimately, the work of the unit would include substantial programmes on native fauna.

The Australian vertebrate fauna is an important one. It includes most (119 species) of the world's marsupials, many bats and rodents, over 700 species of birds and over 390 species of reptiles. There are numerous endemic forms.

Research on the native fauna was certainly needed. The main era of inventory and of collection of specimens was over. There was some taxonomic research proceeding and there was an awakening interest in some State Authorities but, by 1949, there had been few programmes of research on the biology of native animals in the wild. This lack of data on the biology of the animals precluded effective management of them. Meanwhile agricultural and industrial developments continued to alter and to destroy the habitats and the populations of indigenous animals.

The Division of Wildlife Research has no direct responsibility to develop management plans for wildlife populations or to administer legislation about the protection or control of wildlife. In the various States of Australia and in the Australian Capital Territory and the Northern Territory these matters are the responsibility of local departments concerned with fauna conservation and the control of vertebrate pests. To ensure that the research findings of the Division are quickly made available to these departments, however, it maintains close contact with them, both informally and through liaison committees. This is a mutually helpful relationship since much of the Division's work has to be done in the field and depends on collaboration provided by the local departments.

The Division's major role in relation to vertebrate fauna is to provide information about the distribution, status, ethology, ecology and the population dynamics of these species. This provides a sound basis for the development of philosophies, strategies and tactics for management of the wildlife. The objectives of the management range from the preservation of rare species, such as the noisy scrub bird, *Atrichornis clamosus*, in the south-west of Western Australia, to the exploitation and harvesting of game animals, such as various species of duck, and to the control of populations of pest species, such as the the European rabbit, *Oryctolagus cuniculus*.

The Division's field work is integrated with complementary studies of animals in experimentally-controlled laboratory and enclosure conditions at the headquarters in Canberra, and at the Division's research centres in Perth, Darwin, and until recently, Alice Springs.

The Division's work on the rabbit is an example of its studies on a pest mammal. As this is the most important pest mammal in Australia it is fitting that a lot of attention has been given to it. The work has led to major practical advances in the control of the pest, and by clarifying our basic understanding of its biology and ecology has provided a substantial base for the development of more effective policies for control.

The initial plan for the study of the rabbit was for a long term wide ranging programme of investigation into the biology and ecology that might reveal 'chinks in its armour'. But a preliminary investigation of the distribution and status of the rabbit and of the damage it was causing in the agricultural and pastoral industries at the time indicated that there was then a more urgent need for shorter term studies of various control methods.

One of these methods was that of biological control through the use of myxoma virus which had already been investigated by the Division of Animal Health from 1936 to 1943 with generally discouraging results. In 1950 further field trials were started by the Wildlife Survey Section but this time they were in areas of higher rainfall. This led, late in 1950, to a mosquito-borne major epidemic spread of myxomatosis and, within a few months, rabbit populations over a wide area of south-eastern Australia had been drastically reduced by it[1].

For the next few years the Division studied in detail the disease and its vectors, its effect on rabbit populations, and changes occurring in the relationship between the virus and its rabbit host. This work was in collaboration with other divisions of CSIRO, and with the Australian National University, and with State departments involved in rabbit control. It became clear, as had been anticipated, that myxomatosis was not the panacea to the rabbit problem. Strains of virus, less virulent than the introduced strain and better adapted to survive in competition with the more virulent strains, had appeared in the field. There was also evidence of the development in the rabbit of a degree of inheritable resistance to the disease.

The Division investigated other methods of control, particularly that of poisoning, and as a result of screening tests showed that compound '1080' (sodium fluoroacetate) was potentially useful. Its use on a practical scale was then developed in collaboration with State departments and it became the main weapon of rabbit control on many thousands of holdings. Meanwhile the Division developed a series of studies of factors affecting the success of control operations which involve the use of poisons. This work led to recommendations about the technique of poisoning—about the type of bait material; about the placing of the bait; about the timing of the prefeeding before poisoning; and about the strategic timing of poisoning in relation to the time of the year when the rabbits were breeding. Possible side effects on non-target species were also being studied.

The Division then reverted to its original plan and, in 1961, began a series of long term studies of rabbit populations in a range of environmental conditions to determine the interrelationship of the various factors that affect the size of rabbit populations—weather, disease, predators. This has led to a better basis for integrating controls imposed by man with those occurring naturally.

The importance has been highlighted of the population remnants surviving after control efforts, and it is now clear

Birth of a Red Kangaroo. The new-born animal, 1.3 cm long, is about to enter the mother's pouch.

that in the past much control work was a poor investment because the degree of control was not high enough and little was done to prevent the residual populations from increasing. In some arid areas it has been demonstrated that definable survival areas—warrens in favourably situated sites—are of major importance. During a period of drought when rabbit populations are low or even nil over wide areas the survival areas harbour the nuclei from which populations may build up rapidly after drought. The strategic applications of control measures in these survival areas during droughts could lead to long term effective control over large areas.

These studies have also suggested that there is further scope for biological control of rabbit populations by introduction of internal parasites which do not occur at present in rabbit populations in Australia. The Division has recently begun studies of this possibility.

Several other animals have been considered pests of crops and pastures. Because of this, primary producers devote time and money to attempts at controlling them. State pest control authorities have not had the data to help them assess the true pest status of these species or to suggest management procedures. In the past the Division has paid a good deal of attention to these problem animals but now the need is declining and such work will form a less important part of the programme. The 'problem' animals that have been studied include several waterfowl, Anatidae; the Tasmanian waterhen, *Tribonyx ventralis*; wedge-tailed eagle, *Aquila audax*; galah, *Cacatua roseicapilla*; white-tailed black cockatoo, *Calyptorhynchus baudinii*; raven, *Corvus* spp.; emu, *Dromaius novae-hollandiae*; euro, *Macropus robustus*; red kangaroo, *Megaleia rufa*; grey kangaroo, *Macropus* spp.; and dingo, *Canis familiaris dingo*. In each case a substantial study of the life history has been made and at the same time the pest status of the animals has been established.

As one example the large kangaroos, *Megaleia rufa*, *Macropus robustus*, *M. fuliginosus* and *M. giganteus*, have been studied in the field and laboratory. The work has led to an understanding of population changes, movements, reproduction, food and growth and the effects of environmental influences on them. A secure base of data is now available on which management plans can be based. These animals are subject to pest control operations and, more recently to, a high degree of commercial harvesting and doubts have been expressed as to the future of the species.

The Division now believes the animals can well withstand harvesting provided management plans are based on the biological data that are now available.

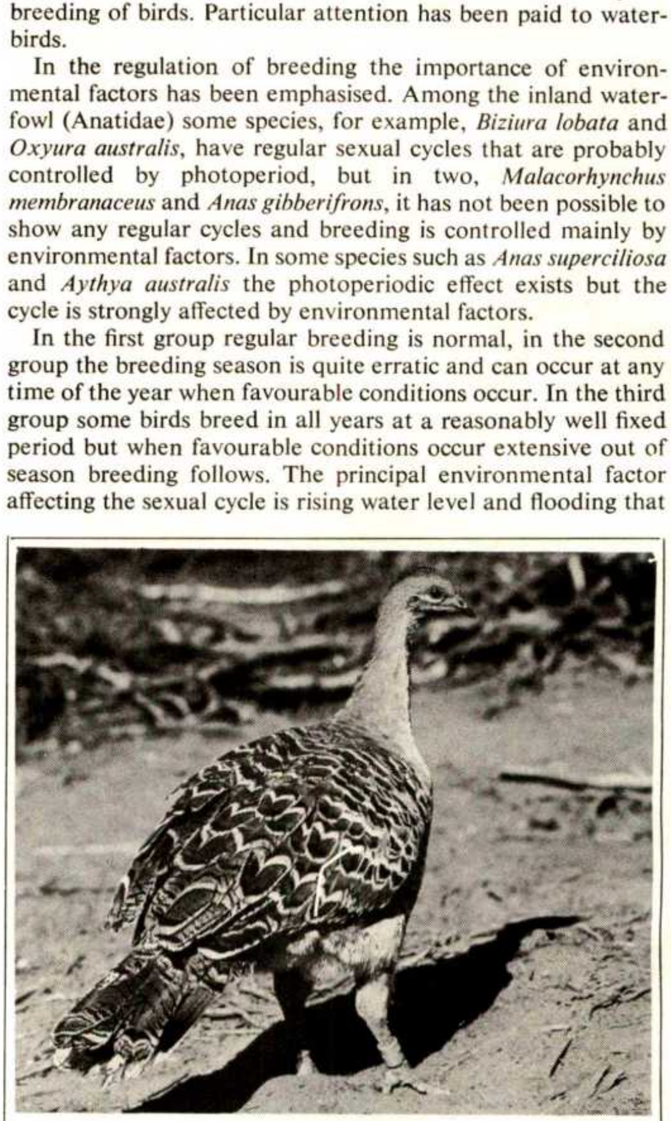
Of particular significance was the finding that populations of some (if not all) species of large kangaroos, flourish on the disclimax plant communities that are produced by domestic stock grazing on native habitats. In severe drought, stock and kangaroos do compete for food but in most years the competition is not great because the two groups of animals tend to select different food items. It is suggested that much land would have greater productivity if both stock and kangaroos were grazed, for harvesting, than if it were devoted to grazing by only stock or kangaroos.

In these studies of 'problem' animals basic biological data accrue, for example, reproduction processes in marsupials, social structure of canids, physiological adaptation to arid zones by euros. In addition, there are other, unexpected results. The study of kangaroos led to the discovery of a previously unrecognised second species of grey kangaroo, *M. fuliginosus*. The discovery was based on observed differences in blood proteins and in morphology, physiology and behaviour. In the study of ravens a fourth species, *C. mellori*, was identified on the basis of behaviour and later shown to have distinctive plumage, movements and food habits. In the white-tailed black cockatoo, two forms (perhaps species) have been shown to exist; they differ in skull and bill characteristics, distribution and ecology. In addition to the intrinsic biological value of these discoveries they have significant implications in management.

The range of climates in Australia and, in particular, the extensive arid and semi-arid zone has encouraged considerable attention to be given to the study of the factors controlling the breeding of birds. Particular attention has been paid to waterbirds.

In the regulation of breeding the importance of environmental factors has been emphasised. Among the inland waterfowl (Anatidae) some species, for example, *Biziura lobata* and *Oxyura australis*, have regular sexual cycles that are probably controlled by photoperiod, but in two, *Malacorhynchus membranaceus* and *Anas gibberifrons*, it has not been possible to show any regular cycles and breeding is controlled mainly by environmental factors. In some species such as *Anas superciliosa* and *Aythya australis* the photoperiodic effect exists but the cycle is strongly affected by environmental factors.

In the first group regular breeding is normal, in the second group the breeding season is quite erratic and can occur at any time of the year when favourable conditions occur. In the third group some birds breed in all years at a reasonably well fixed period but when favourable conditions occur extensive out of season breeding follows. The principal environmental factor affecting the sexual cycle is rising water level and flooding that



The Mallee Fowl, *Leipoa ocellata*, an Australian inland bird that does not brood its eggs but buries them in mounds of sand and vegetation.

can occur in locally dry conditions due to rain on distant catchments.

The work on reproduction is supported by studies of food habits, behaviour and movements. The demonstration of the erratic breeding seasons and the demonstration of widespread nomadic movements and the explanation of their causes help State fauna conservation authorities to devise appropriate management procedures.

The Division now believes that its principal contribution to the biological base of waterfowl management is concluding and is moving emphasis to the graniverous dry land birds of the arid and semi-arid zone. The breeding of all arid zone pigeons and the stubble quail, *Coturnix pectoralis*, are under study. Preliminary results show that all of the arid zone pigeons and the quail have regular annual sexual cycles but, with few exceptions, there are strong effects of rainfall on those cycles and on the breeding seasons. There is reason to believe that the rainfall operates through the availability of food and hence the nutrition of the birds. A similar explanation was suggested for the effect of changes in water-level on the cycles of waterbirds.

In the well-watered tropical north although the breeding seasons of waterbirds are, superficially, regular they are to a large extent controlled by changes in water level, as in the arid zone. In some species, for example magpie goose, *Anseranas semipalmata*, the effect of water level that is important seems to be the density of swamp vegetation available for nesting. Graniverous pigeons of the tropics, as in the arid zone, have breeding seasons largely controlled by rainfall and its effects on food supply.

Management of populations of many animals is not possible without precise data on their movements and other biological attributes that can only be collected from individually marked animals in the wild. For that reason, in 1953 the Division set up the Australian Bird-Banding Scheme and in 1961 the Australian Bat-Banding Scheme. These are operated as services. In addition to the Division's own staff, university staff, State authorities and amateurs can become registered banders on approval of their proposed projects and their competence to band. Bands, other equipment, office support and advice are provided; copies of all data are kept in the Scheme's archives. In 1975 there were 338 registered banders. Since 1953 1,270,000 birds of 780 species have been banded in Australia and its Territories and 270 papers have been published utilising results of the banding scheme. In the more specialised Bat-Banding Scheme there are now 12 registered banders and 65,000 bats have been banded.

Some elements of the Australian fauna, particularly the ground-living mammals had declined before settlement and the effects of introduced grazing animals and predators. In vast areas of the continent there is no real idea of the present status of many forms. For these reasons a survey group was established in 1962.

Regions for surveys are selected because of the imminence of development or because of their intrinsic faunal interest. They are visited by parties comprising ornithologists, mammalogists, herpetologists and botanists. Each region is visited several times at different seasons. Data on the distribution, present status, habitat needs and other aspects of the biology of many species are collected. The planning of conservation reserves and their ultimate management can proceed on the basis of these data, and conflicts with mining and other developments can be viewed in better perspective.

The survey group has worked in New Guinea and continues to work in southern Australia but now believes that its main role is in the sparsely settled north of the continent and is concentrating on Arnhem Land at present. In later years it is planned to expand both east and west from that area.

In addition to what might be called the predictable results many surveys produce novelties; animals considered very rare or extinct are discovered in some numbers (for example, the pygmy possum, *Burramys parvus*; the delicate mouse, *Leggadina delicatula*; the Australian native mouse, *Pseudomys, novaehollandiae*; the marsupial mice, *Antechinus bilarni* and *A. bellus*; the rock rat, *Zyzomys woodwardi*; and the Kangaroo *Macropus bernardus*). Nests and eggs of some birds are described for the first time (for example, *Ptilinopus alligator*, *Petrophassa rufipennis*, *Amytornis woodwardi*) and the known ranges of many animals are greatly extended.

An Echidna, *Tachyglossus aculeatus*, just hatching from its egg. The Echidna and Platypus, both monotremes, are only found in Australia.

In addition to the formal survey and censusing of wildlife in various regions the Division is planning to develop methods of rapid survey and evaluation of fauna in the whole continent. Work has begun, in conjunction with other Divisions, to establish methods to classify and to rapidly assess the value to aquatic fauna of the wetlands of the continent. It is hoped to begin soon to do similar work with dryland habitats and so provide the faunal component to the long term Ecological Survey of Australia that is under discussion.

Much of the work of the Division to date has been concerned with single species or with groups of species of animals. The Division has been aware for some years that major advances could be made by the examination of whole communities and it plans to develop this work more in the future. The first such study became possible when a laboratory was constructed in Darwin, Northern Territory in 1971 and a group of mammalogists, ornithologists and botanists was established there. The aim is to study the productivity and management of tropical grasslands and their fauna, particularly as these are affected by the introduced water buffalo, *Bubalas bubalis*, and feral pig, *Sus scrofa*.

The group now has been given control of a large study area wherein field laboratories are to be established, on the South Alligator River. In the first phase of the group's work the ecology of some of the more important animals and habitats has been studied. The next phase is now beginning and the work of each person will be closely integrated into the main theme. Considerable opportunities will exist for visiting scientists to participate in this programme.

Most of the work of the Division has been ecological or behavioural and studies of physiology have been used mainly in a supporting role although there have been exceptions, such as fundamental physiological, anatomical and biochemical research on the echidna, *Tachyglossus aculeatus*, and on the platypus *Ornithorhynchus anatinus*, reproduction in kangaroos and olfactory communication in rabbits. Recently, in line with the Division's increasing role in research on the native fauna for its own sake, a physiological group has been established that will undertake long term research on the fundamental physiology of marsupials.

The future trends in wildlife research in CSIRO can be summarised as increasing attention to research by multidisciplinary groups on communities rather than on groups of species or single species and increasing attention to research, including surveys of fauna in remote regions, particularly in tropical Australia.

I thank B. V. Fennessy for help in the preparation of this article.

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[1] Ratcliffe, F. N., *et al.*, *Nature*, 170, 7 (1952).

Animal nutrition to human nutrition: achievements and prospects

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The Division started in 1927 as one of Animal Nutrition, particularly interested in the role of trace elements in sheep. Progressive changes in title of the division clearly show the way emphases have changed, until now, although there is still much animal work being done, new programmes are being planned with particular relevance to human welfare. Some of this work will be in biochemistry and physiology, but the division is also committed to ecological and behavioural approaches to human nutrition.

IN 1927 the Council for Scientific and Industrial Research (CSIR) established a laboratory in South Australia at the University of Adelaide with the intention that it be involved in research into animal nutrition with particular relevance to problems concerning the agricultural and livestock industry of Australia.

During the ensuing years this laboratory, first as the Division of Animal Nutrition (1927–33), then as part of the Division of Animal Health and Nutrition (1934–44) subsequently as the Division of Biochemistry and General Nutrition (1944–65) and, finally, the Division of Nutritional Biochemistry (1965–1975), became well known for its contribution to various aspects of ruminant metabolism, with particular emphasis on the role of trace elements. Successive Chiefs were Professor T. Brailsford Robertson (1927–30), Sir Charles Martin (1931–33), Dr L. B. Bull (1935–44), Dr H. R. Marston (1944–65) and Dr A. T. Dick (1965–75).

When, in 1975, a decision was made to establish a research centre in Australia for work on human nutrition within the structure of CSIRO it was opportune to redirect the activities of the Division of Nutritional Biochemistry and incorporate much of the expertise and experience built up over many years of research in animal nutrition into a new Division of Human Nutrition.

Since Dr B. S. Hetzel, formerly Professor of Social and Preventive Medicine, Monash University, was appointed Chief, the Division of Human Nutrition has begun to develop research programmes with particular relevance to human welfare. Some of these are a natural extension of areas covered in earlier work with sheep. The background of past achievements provides a challenge for the future of the new Division. Initially three main programmes in human nutrition are planned—trace elements and mineral metabolism, metabolism and digestion, epidemiology and human ecology. The achievements of the past and the prospects of the future will now be reviewed.

Research in trace elements

A major concern of the Division for more than 40 years has been the role of trace elements in the nutrition of sheep. This interest stemmed from the realisation that animal husbandry in extensive, well-watered coastal tracts in the comparatively arid state of South Australia had been rendered precarious from the earliest days of settlement by the uncertainty of pasture and crop production and the risk of Coast Disease in grazing sheep and cattle. Sheep restricted to these regions commonly developed a profound anaemia, lost appetite progressively and died in an emaciated condition within a matter of months.

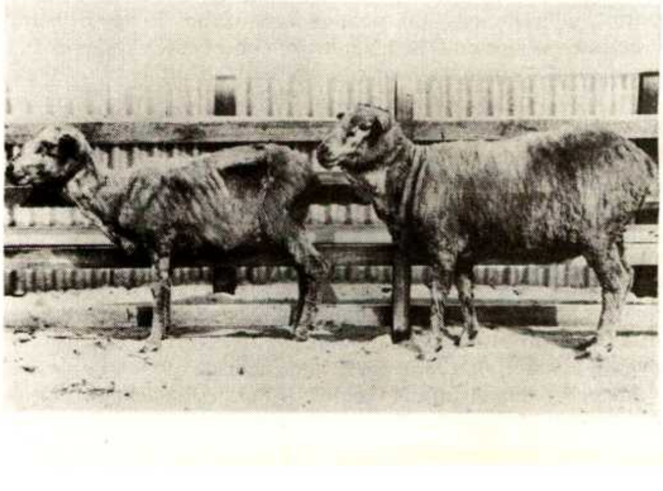
In 1928 Sir Arnold Theiler, then Director of the Onderstepoort Veterinary Research Institute had been invited to visit Australia and advise the newly established Council on profitable avenues of animal research. He suggested that Coast Disease in sheep, in which bone fragility was a common feature, might well be due to phosphate deficiency. This hypothesis was disproved experimentally and, in the early thirties a Coast Disease Committee was formed by Sir Charles Martin to coordinate the systematic study of the disease.

The highly calcareous affected soils were composed almost exclusively of comminuted shell fragments blown from the continental shelf exposed during the last Ice Age and it was predicted that they would lack not only phosphate but also traces of heavy metals. Some of these (for example, iron and copper) were already known to be essential animal nutrients.

Sheep offered a lick containing small amounts of seven metallic elements, including cobalt, showed a limited response and on the basis of a German report that cobalt could induce polycythemia in rats, the possibility that cobalt deficiency might be intimately associated with the anaemia and other features of Coast Disease was explored. Pure cobalt nitrate given at the rate of 1 mg Co/d elicited dramatic responses in coasty sheep at pasture or in pens (Fig. 1). Subsequently, copper deficiency was shown to complicate the primary deficiency of cobalt. An historic account of these events appeared in 1938 (ref. 1).

Cobalt proved to be effective only within the rumen[2] but the cumbersome necessity to provide cobalt supplement by frequent drenching or other means was eventually overcome by exploiting a suggestion by a visiting Fulbright Research Fellow, Professor Perry R. Stout, that a device might be lodged in the sheep's rumen to release cobalt slowly and continuously. A heavy pellet, comprising cobaltic oxide and iron powder, was evolved

Fig. 1 Merino ewes depastured on grossly deficient shell-sand dunes at Robe, South Australia. The emaciated ewe (left) has been given copper but is suffering the final stages of cobalt deficiency; the comparable healthy animal (right) has been adequately dosed with both cobalt and copper.



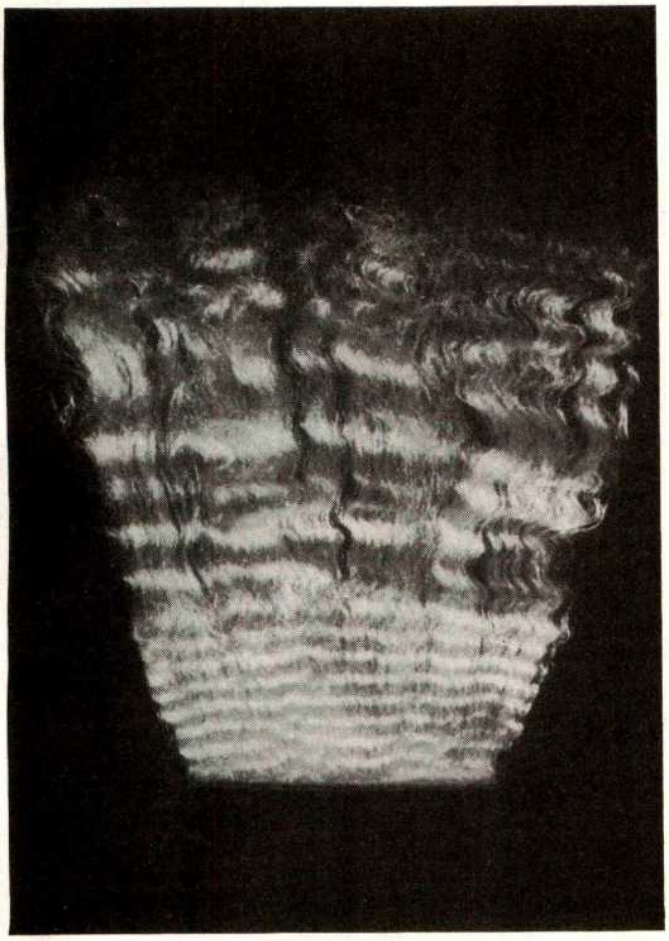


Fig. 2 Effect of copper deficiency on Merino wool[8]. The copper-deficiency lesion is apparent in the upper part of the staple grown by an acutely deficient ewe. Restoration of adequate copper status resulted promptly in normal crimp in later growth.

in the Division which, given with a steel grinder to reduce a tendency for calcium phosphate to encrust the pellet, satisfies these requirements for Merino sheep in most circumstances[3].

In sheep maintained by cobalt supplements, the effects of copper on a specific deficiency lesion in wool (Fig. 2), on wool growth, fleece pigmentation, anaemia and growth were studied intensively[4] and led, early, to the application of copper fertilisers with spectacular results[5] to crop and pasture species which had invariably failed on these recalcitrant soils (Fig. 3). The successful development of large tracts of soils derived from the calcareous sands was made possible. Cereal crops, improved pastures and healthy flocks flourish where only stunted scrub had previously grown, in spite of an adequate rainfall.

Space does not permit reference to studies with selenium[6] and other minor and major elements except to mention that the principles involved in the useful development of the cobalt pellet have been applied to the production of a selenium pellet[7] which provides a ewe and her suckling lambs with their selenium requirements for several years and so prevents the occurrence of White Muscle Disease in lambs in deficient areas.

Many aspects of these researches, which changed the face of agriculture in South Australia and have been widely applied elsewhere, have been reviewed by Marston[8,9].

In 1948 came the recognition[10,11] that vitamin B_{12}, the newly isolated anti-pernicious anaemia factor, contained cobalt. Within a short time workers at Cornell[12] had shown that cobalt-deficient sheep responded to injected vitamin B_{12}. This finding was quickly confirmed in the Adelaide laboratory[13] and it became apparent that the sole function of cobalt in the rumen was to enable the microflora to synthesise vitamin B_{12} (ref. 14). There followed a series of metabolic[15,16] and biochemical[17,18] studies into the nature of the disease.

The most prominent effects in sheep are depression of appetite, inefficient use of food and severe depletion of the stores of folic acid in the liver. Most of these effects can be attributed to depressed activity of two vitamin B_{12}-containing enzymes. The first of these[17] is methylmalonyl-coenzyme A (CoA) mutase which catalyses the reaction

$$\text{methylmalonyl-CoA} \leftrightharpoons \text{succinyl CoA}$$

one of five sequential steps in the conversion of propionate into succinate. Ruminants derive a substantial part of their energy from propionate produced in the rumen and its accumulation in the systemic blood appears to produce the loss of appetite so characteristic of the disease[16]. The second critically affected enzyme[18] is the methyl-transferase that catalyses the reaction

$$\text{5-methyltetrahydrofolate} + \text{homocysteine} \leftrightharpoons \text{methionine} + \text{tetrahydrofolate}$$

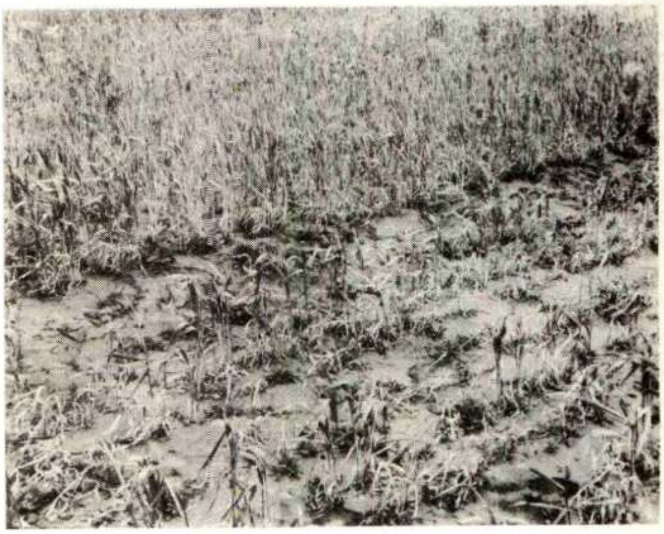
Failure of this reaction to occur leads to a deficiency of available methionine. The lack of methionine is responsible for the appearance of fatty liver and may limit body growth. It is also responsible for the depletion of liver folates[19].

The appearance of megaloblasts in human pernicious anaemias is generally attributed to impaired synthesis of DNA due to a lack of 5, 10-methylenetetrahydrofolate. The severe depletion of folate stores that occurs in vitamin B_{12}-deficient sheep is not accompanied by delayed maturation of red cells, however, and further work to study this aspect of human pernicious anaemia will be carried out in the monkey.

Recent work in the Division on copper deficiency has been directed at the disease of young lambs known as enzootic ataxia or swayback. The condition is characterised by degeneration of myelin in the spinal cord and may be prevented by dosing the pregnant ewe with copper. Current work is concerned with the biochemical mechanism by which copper exerts its effect on development of the central nervous system of the foetal lamb. These studies may prove to be relevant to the degenerative brain disease of children known as Menkes' kinky hair syndrome[20] which has been traced to a systemic deficiency of copper.

The multidisciplinary approach to the resolution of trace element deficiency diseases in animals may be appropriately directed to problems in human nutrition. Epidemiological

Fig. 3 Copper response in wheat[5]. Wheat grown on calcareous sand at Robe, South Australia. Foreground, superphosphate only; background, superphosphate *plus* copper sulphate.



studies precede the recognition of a nutritional deficiency and define the problem as with Coast Disease. An intensive study of physiological or microbiological implications follows and, finally, as in the case of cobalt, definition of the precise function of the missing nutrient is reached in biochemical terms.

One of the most widespread and certainly the most obvious of trace element deficiencies in man is that of iodine, and work related to iodine deficiency in the human population will form one of the major new studies in the reconstituted Division. Extensive epidemiological work has already been done[21,22].

Endemic goitre and the associated condition of mental deficiency, deaf-mutism and spastic diplegia known as endemic cretinism has been recognised in Europe, and especially in the Alps, for centuries.

Studies in New Guinea[23] and elsewhere revealed that endemic cretinism (Fig. 4) was associated with a more severe iodine deficiency than was endemic goitre. Iodine intake was usually less than 20 $\mu g\ d^{-1}$ as compared with a normal intake of about 100 $\mu g\ d^{-1}$.

A long standing controversy as to whether endemic cretinism was related specifically to dietary iodine deficiency led Hetzel, Buttfield and Pharoah to set up a controlled clinical trial in the highlands of New Guinea to determine the effectiveness of a single injection of iodised oil as a long term prophylactic. By 1970 it was apparent that effective prevention had been achieved provided the mother had received an injection of iodised oil before, and not during, pregnancy[24]. Extensive mass injection campaigns have now been planned and carried out in a number of countries including New Guinea, Nepal, Peru, Ecuador and Zaire.

From the results of the trial, iodine seems to have exercised a specific effect on the early development of the central nervous system, perhaps during the phase of early neuroblast multiplication from 10–18 weeks[25]. This phase of brain growth predates the achievement of full competence by the foetal thyroid gland and raises the question of whether thyroid hormone or iodine itself may be responsible for protection against the disease[24]. Experimental work to study the mechanisms involved at the physiological and biochemical levels is being actively pursued in the new Division, using first the sheep and later the monkey as the experimental model.

Metabolic studies

During the period 1939–46 research into the digestive processes within the rumen of sheep by a group at Cambridge[26] and by Marston[27] and Gray[28] at the CSIR Animal Nutrition Laboratory established the importance of microbial degradation of fodder to short-chain fatty acids in the rumen.

The three main fatty acids (acetic, propionic and butyric) produced in the rumen and absorbed from this forestomach were shown to provide much of the energy requirements of sheep. It was also established that ruminants, unlike monogastric animals, absorb virtually no glucose as such from the alimentary tract. They rely on gluconeogenic processes in the liver for their supply of glucose by conversion of propionate absorbed from the rumen, and of amino acids derived from microbial protein and absorbed from the intestine.

This work established the pattern for much of the subsequent research in animal nutrition and demonstrated the relevance of basic research to such agricultural problems as drought feeding, efficiency of utilisation of pasture and pregnancy toxaemia.

While one group[29,30] was elucidating the effects of dietary factors on the production and relative proportions of short-chain fatty acids in the rumen, another group[31–33] was studying the intermediate processes involved in the utilisation of acetate for energy requirements and of gluconeogenic precursors for glucose production.

During this period (1950–65) many of the questions being asked about the efficiency of utilisation of different types of fodder were being resolved by microbiological and physiological attacks on the problem. There was, also, a growing interest in enzyme mechanisms and the control of metabolic pathways in ruminants[34]. This work, while continuing to lay down foundations for further understanding of ruminant nutrition, led to a more acute awareness of biochemical differences between ruminants and monogastric animals, particularly in relation to the significance of gluconeogenic and lipogenic pathways.

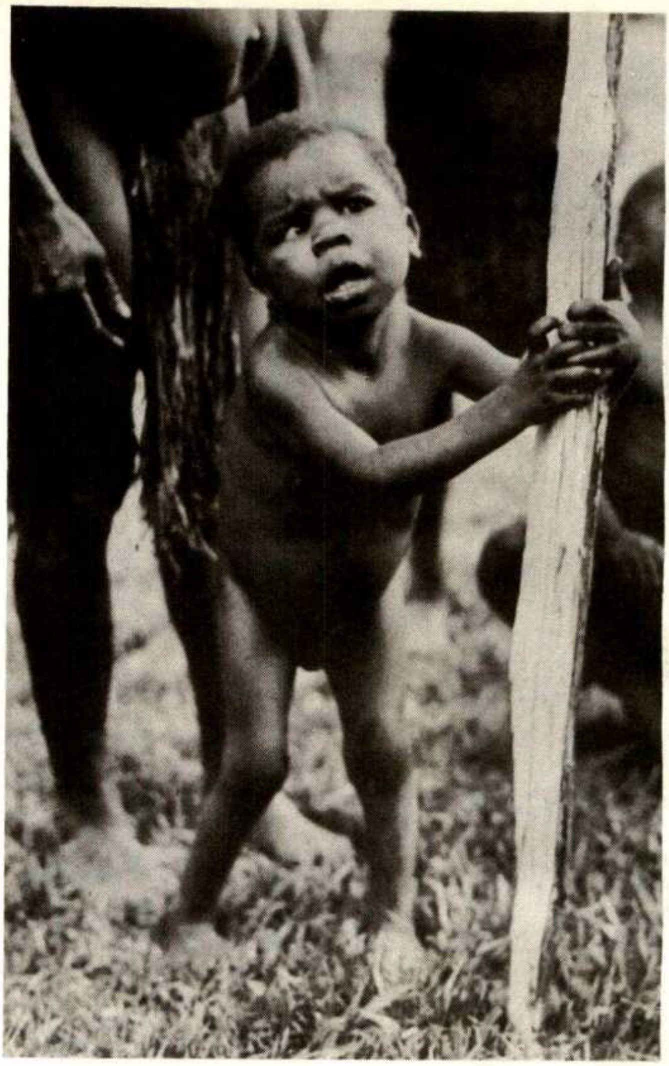


Fig. 4 A young New Guinea boy with cretinism, showing squint, vacant facies and leg deformities[21]. Permission has been granted by the editors of *The Medical Journal of Australia* for the publication of this photograph.

It was shown elsewhere[35] that ruminants lack two important enzymes involved in a lipogenic pathway from glucose in liver and adipose tissue. Hence sheep normally rely on acetate as a lipogenic precursor rather than on glucose which is the major source for fat synthesis in monogastric animals.

Later studies in the Division proved that these two enzymes are adaptive[36]. In newborn lambs reared on a high carbohydrate diet or in adult sheep receiving large amounts of glucose administered intravenously or into the abomasum (and thus by-passing the rumen) these enzymes are present. Glucose can then act as a lipogenic precursor and in this respect sheep behave metabolically like monogastric animals.

The expertise acquired in animal nutrition is now being applied to the three new metabolic programmes envisaged for the Division—digestion and gastrointestinal disorders; efficiency of utilisation of food stuffs and metabolic aspects of obesity; protein metabolism, degradation and turnover.

With the growing concern about the preference for highly refined foods in Western society and the epidemiological correlations reported for related gastrointestinal disorders, an experimental study will be made of the effects of dietary fibre in monogastric animals. Various components of fibre, their relationship to water absorption, bile salt degradation and rate of passage of digesta will be examined. A collaborative programme with a Clinical Gastroenterology Unit is being planned.

Fundamental studies on protein degradation using isolated cell preparations have been actively pursued[37]. Measurements with cultured cells were commenced to establish a model system in which various hormones, nutrients and other compounds could be tested for their effects on general protein degradation and on proteolytic pathways for the removal of error proteins. These studies will be applied to problems relevant to wasting diseases, growth and ageing.

Work currently being undertaken with isolated hormonally responsive rat liver cells on interrelationships between high carbohydrate and high fat diets, metabolic pathways and hormone efficacy will be integrated with whole animal studies on metabolic and hormonal aspects of obesity.

Human nutrition in an ecosystem

In its pursuit of solutions to problems in human nutrition, the Division will be taking a further conceptual step—denoted by the concept of the ecosystem. The application of this concept will now be considered in relation to two urgent nutritional problems in Australian society—the nutritional state of the Australian Aborigines and the problem of alcohol consumption.

An ecological approach is urgently required for a distinctively Australian problem in human nutrition—the nutrition of the Aborigines. Gross malnutrition, particularly of infants, is very common among the Aboriginal people. It is a major cause of the unacceptably high infant death rate in the first year. Compared with an infant mortality of 20.7 per 1,000 live births for white Australians in 1967–69, Aboriginal infant mortality in 1965–69 averaged 112 for the Northern Territory[38]. This gross malnutrition has resulted from complete disruption of the normal pattern of living which represented a reasonably satisfactory adaptation before the arrival of the white man[39,40]. Loss of tribal lands and contact with white Australian society has been accompanied by cultural disintegration leading to loss of motivation for hunting and the tribal way of life. One by-product of this is malnutrition in infancy, another is alcoholism in adults. Improvement may come from an approach designed to develop a viable ecosystem, as independent of white society as possible, to give the Aborigine time and opportunity to reintegrate with his environment.

There is a challenge to Australia to do something much more imaginative for the Aborigine than has been done so far. CSIRO has much to offer here, concerned as it is with new approaches to energy and water conservation, soil vegetation and feral animals, as well as nutrition and housing.

Alcohol consumption is rising progressively in Australia as in other developed industrial urban nations. Surveys of levels of consumption reveal 6.5% in excess of an average of 100 ml d^{-1} of absolute alcohol in 1971 compared with 3.4% in excess of this level in 1949. The level of 100 ml d^{-1} is generally considered a high risk level for a traffic accident, serious physical disease and death before the age of 50 yr. Emphasis is being laid on levels of alcohol consumption rather than on "alcoholism" because of the need to intervene much earlier in the natural history of the disorder to have some chance of successful treatment and rehabilitation. An elevated blood alcohol is found in more than half of the victims of fatal traffic accidents and the numbers are increasing each year. Excessive alcohol consumption is a significant factor in at least 20% of hospital admissions and a major factor in family breakdown and serious crime.

Alcohol consumption is increasing most in the young adult group, especially in young men. This phenomenon is clearly related to the social environment—the high social approval of heavy drinking, the promotion of drinking in association with sport and a masculine out-of-doors image. The lack of adequate recreation and education for leisure in an affluent society accentuates the problem. There is an urgent need for research to understand the genesis and maintenance of the life style of the young adult with a view to development of a suitable social strategy to reduce alcohol consumption[41]. An ecological approach with special attention to family, school, peer group and mass media is clearly indicated. Education, legislation and new services will all have a place in any such social strategy. Furthermore, alcohol intake should not be isolated from other aspects of diet such as carbohydrate and fat intake or life style including cigarette smoking, working and leisure patterns.

Conclusion

In considering this transition of a Division of Animal Nutrition into a Division of Human Nutrition, we have to recognise that much more is known about the basis of animal nutrition because of the better opportunity for experimentation. Human nutrition needs to rely on animal models for final clarification of mechanisms. The application of chemical, biological and behavioural approaches depends however on adequate epidemiological study in the first place. Once this definition has been achieved, means will need to be devised for appropriate modification of human dietary patterns—a difficult task as indicated by existing problems in modifying cigarette consumption in spite of overwhelming evidence of relevance to lung cancer.

The Division is therefore committed to an ecological and behavioural approach to human nutrition along with the more traditional disciplines of biochemistry and physiology. Only with a total view of man can adequate understanding be achieved.

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Growth of CSIRO's interests in food research

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In 50 years CSIRO has developed its interests in food research from a few projects on urgent technical problems confronting the export trade to an organisation with three large laboratories engaged in broadly-based programmes of basic and applied science.

On the second day of its first meeting (April 14, 1926), the Executive of the Council for Scientific and Industrial Research (CSIR, later the Commonwealth Scientific and Industrial Research Organisation, CSIRO) drew up a list of the "urgent and promising" lines of investigation on which it considered immediate scientific effort must be concentrated. One of the five subjects listed was food investigations; after some discussion the members of the Executive divided this into research on the problems of dairy manufacture and research into food preservation, with particular attention to problems of cold storage. The need of industry for such scientific and technical help had been uncovered by failures in Australia's important export trade in foodstuffs, which was worth $A25 million in 1926 and is today worth more than $A1,000 million. "Industry that relies solely on the scientific work of other countries", so the Executive judged, "cannot hope to occupy a commanding position in the world market". Within Australia too, the normal problems of transport and long term storage of perishable foods were exaggerated by the tremendous distances and the uneven distribution of population. CSIR's interest, however, was only incidentally in industry's housekeeping problems; its primary aim was to increase the sum of knowledge on food and food constituents. Research was to be of a fundamental nature, and on problems that transcended state boundaries and required the long term attention of a team of specialists.

From such beginnings grew one of the largest organisations in CSIRO. The present Division of Food Research has some 350 people working for it, of whom about 40% are professional scientists; the Australian Government provides about 80% of its budget, exceeding $A4 million a year, with most of the remainder contributed by the meat and dairy industries. This Division, which resulted from the amalgamation of the Division of Food Preservation and the Division of Dairy Research, has become the major Australian centre for research studies in food science and technology. It has large research laboratories in four states, each specialising in work on particular foods such as fruit and vegetables, poultry and eggs (New South Wales), meat (Queensland), dairy products (Victoria), and seafoods and potatoes (Tasmania). CSIRO also makes grants to the two trade-directed Institutes of Bread Research and Wine Research, and a few food investigations are carried on in other divisions such as Entomology (storage of bread grain) and Horticultural Research (disorders of fruits).

For an integrated approach to the problems of food science it is necessary to employ workers in many disciplines, such as physics, chemistry, biochemistry, microbiology, engineering and the physiology of plants and animals. Such highly trained scientists, particularly any with special qualifications for food research, were not numerous in Australia during the 1920s. CSIR began by selecting likely-looking leaders from among the young university graduates and sending them to England on scholarships; there they obtained specialist training at the Low Temperature Research Station, Cambridge, or at the National Institute of Dairy Research. The resulting delay in setting up any CSIR organisation for food research could scarcely have been avoided; nonetheless, it had serious consequences. When the first trainees, J. R. Vickery and W. J. Wiley, returned to Australia in 1931 the depression had already hit the country and CSIR was struggling to keep going. Establishment of two new divisions, or of any, was out of the question. Virtually all dairy research was postponed indefinitely and work on food preservation was confined to the two commodities of greatest importance to export—beef and fruit—and had to be carried out in laboratories lent or shared by other organisations. The Section of Food Preservation (a Division from 1940) was established in 1931 with Vickery as leader and seven other research officers. The Section of Dairy Research (Division, 1962) under Wiley could not be formed until 1940.

Depression and the Second World War

The new group's first resounding success came with its first undertaking: it played a central role in initiating the export of chilled beef to Britain, thereby opening a lucrative market to the Australian cattle industry[1,2]. This is probably the longest lived of all the Division's lines of investigation: research on chilled beef is now important for the new packaging and quality requirements of the Japanese and US markets. CSIR had been able to start work on fruit storage and ripening as early as 1927, in collaboration with the State Departments of Agriculture, and the Division's thoroughgoing investigations in this area made it possible to develop a scientific basis for the storage and export of pears, apples and oranges, and to recommend revolutionary new methods for transport and ripening of bananas within Australia.

When the country finally emerged from the Depression in the late 1930s, new and more central laboratories were set up at Homebush, New South Wales, but plans to establish food research on a more comprehensive basis, embracing basic food science studies as well as empirical work on all the important commodities, were foiled by the outbreak of the Second World War.

The war made exceptional demands on Australia's food-growing and food-processing industries, and the Division of Food Preservation together with the embryo Division of Dairy Research in Melbourne were the only centres in Australia where research on food technology was being carried out and where there was a group of people who could advise the Government on problems in this field. Now attention moved away from problems of cold storage to concentrate on methods of preservation that did not require refrigeration, such as artificial skin-coating of fruits, dehydration and, above all, canning. Although Australia had exported canned meat for 75 years, knowledge of the canning of vegetables and fruit juices was sadly lacking. Yet Australia was required to supply a wide range of preserved foods for the armed forces of the USA and Australia in the South-west Pacific area. The technology needed to bring the Australian canning industry into the twentieth century was worked out in the Homebush laboratories, with the willing cooperation of visiting American food technologists and of industry leaders. Similarly, the Division of Food Preservation developed and prepared the first survival ration packs of concentrated foods for the Australian Army. The Division of Dairy Research created a butter with properties suitable for use in the tropical theatres of war. Both divisions acted as service laboratories for the Government, vetting specifications, carrying out regular quality control checks, issuing certificates—all jobs that were anathema to the original concept of CSIR and that were shed as soon as possible.

Food preservation after the war

The staff of the Division of Food Preservation had doubled during the war and the expansion of staff and resources continued. It was now essential to devote more attention to fundamental work to obtain a better understanding of the constituents of foods and their reactions to different environments, and so provide a basis for further advances in the applied field. The division was reorganised so as to devote about 50% of all its resources to food science. Three groups were formed at Homebush, in chemistry, physics and microbiology; there was no room for more in what had become extremely cramped laboratories. By an imaginative pooling of resources with the University of Sydney, which lacked senior research staff as much as the Division lacked space, it was possible to form two new units—the first in Australia—of plant physiology and physical biochemistry, which were located at the university.

All these groups carried out important basic research which earned the Division an excellent international reputation.

The research workers in the Plant Physiology Unit, for example, made notable biochemical and biophysical contributions to the knowledge of the mechanisms of the active transport of cell constituents against osmotic gradients[3]. They greatly increased the understanding of the ripening of apples and bananas, particularly in relation to the effects of ethylene[4].

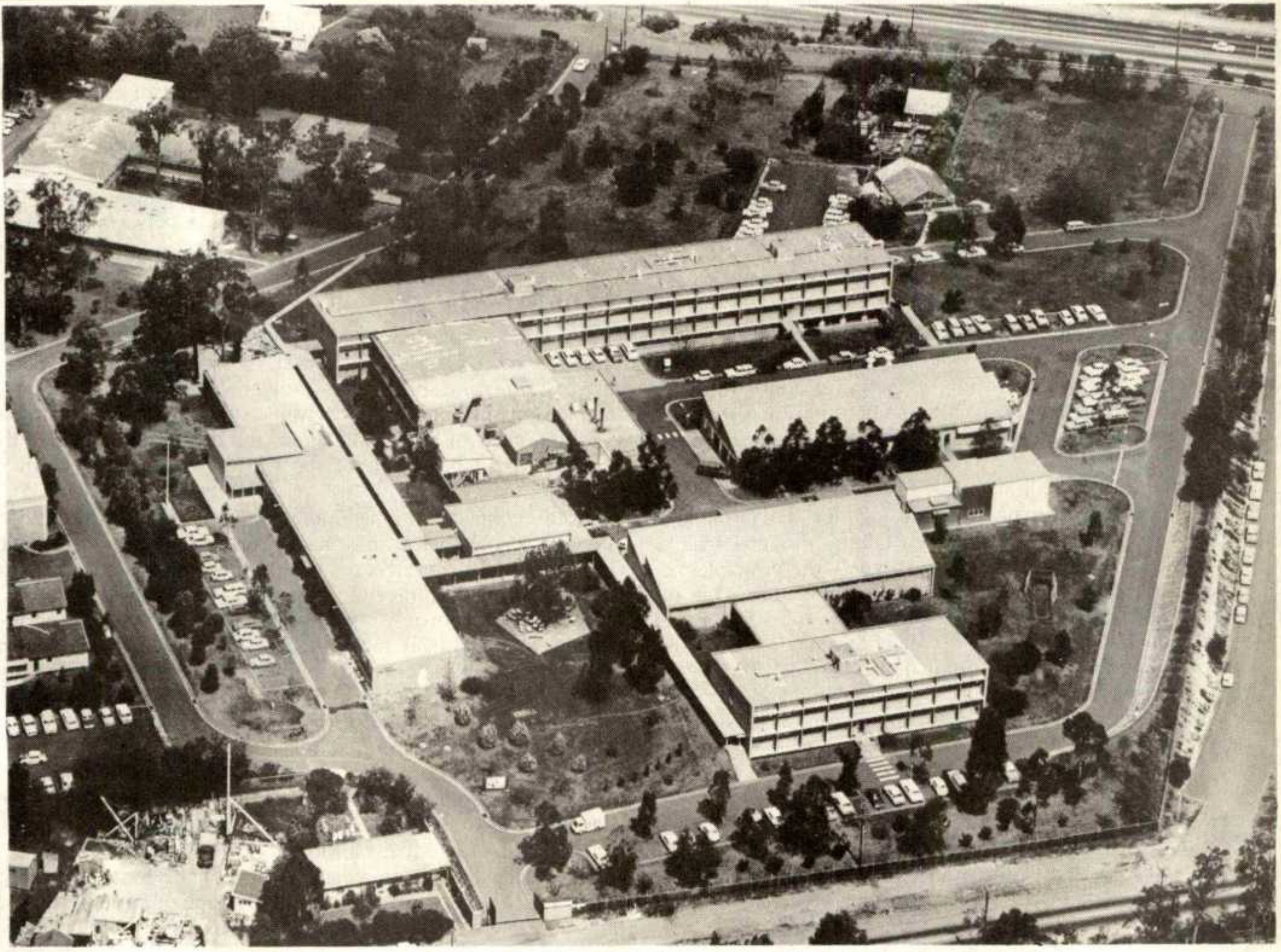
Work by the Physics Section on the evaluation of heat penetration processes in relation to the survival of heat-resistant bacterial spores during canning is one of the classics of food technological research[5].

The scientific basis of preservation by salt and sugar and by drying was firmly established by the results of investigations on the water activities of foods and the way in which they control the growth of microorganisms subjected to a wide range of substrate temperature and composition[6].

Non-enzymatic browning is often a potent cause of deterioration of stored foods. Major contributions to the elucidation of the complex chemical reactions and the role of the inhibitor, sulphur dioxide, have been made by one of the Division's research teams[7].

Some of the advances led to developments in the applied field; for example, scientists at the Division used new knowledge from plant physiology when making a device for the rapid determination of the maturity of peas for freezing or canning.

In 1961, large and well equipped new central laboratories were opened at North Ryde, on the outskirts of Sydney, and four years later special funds from the meat industry permitted the building of a new and much enlarged meat research laboratory at Brisbane. In the decade after the Second World War the Division was successful in placing branch laboratories in regions with special problems: a laboratory which was to grow quickly in strength and effectiveness was placed in the New South Wales coastal area to work on remedies for citrus wastage and fruit-fly infestation; another was placed in a fishing port on the southern coast of New South Wales. A strong unit was set up at Hobart, Tasmania, to give technical assistance to the important local fruit and vegetable industries and fisheries; this unit was called in to advise the State Government recently, when levels of heavy metals in Tasmanian fish and oysters were causing worry to the authorities. After the Second World War, in addition to its research activities, the



Central Laboratories, CSIRO Division of Food Research, North Ryde, New South Wales.

Division helped to establish departments of food science at tertiary institutions in Australia, and in the course of several decades it trained many Colombo Plan and other research students from the developing countries.

When Vickery retired in 1967 he could reflect with both satisfaction and irony that it was probably only in the previous 20 years that the Division had achieved the full stature that the Executive had envisaged for it in 1926. Its activities, however, were still somewhat unbalanced, 40% of research effort being devoted to foods of plant origin and only 25% to foods of animal origin, a state of affairs that scarcely did justice to the importance of animal foods in the Australian economy. After M. V. Tracey, former leader of the CSIRO Wheat Research Unit, became the Division's second chief, the balance was rectified by an infusion of money and workers into meat research, and by the amalgamation of the former CSIRO Division of Dairy Research. By 1971 the corresponding figures were 20% for foods of plant origin and 55% for foods of animal origin, with the balance, as before, comprising basic chemistry and physics, and work applicable to both types of food.

Dairy research up to amalgamation

In some important respects, dairy research is the antithesis of food research generally; it deals with one raw material instead of many and in most cases it investigates products that have already been processed or preserved by age-old methods, whereas food scientists in general deal with a range of raw materials which often need new, or greatly altered, forms of preservation. It seems reasonable, therefore, that dairy research should exist as a separate specialist group while food research is covering some of the essential groundwork, but should be incorporated later into the larger research group so as to take advantage of wider horizons and bigger resources.

During its 30 years of independent existence the Division of Dairy Research had an important role in bringing improved or sometimes radically new processes to industry and in achieving new understanding of the chemistry and microbiology of dairy products. As dairy technology was already fairly advanced and successful, the Division was from the beginning able to concern itself with the relatively sophisticated problems of quality (particularly of flavour) and uniformity of dairy products, with methods of reducing the labour cost of manufacturing operations and with the possibility of creating acceptable new foods from the constituents of milk.

In its early years, led by W. J. Wiley, the group investigated problems of butter, which then used 80% of all milk produced in Australia. It was successful in eliminating serious causes of tainting and in showing industry that some traditional steps of butter making could safely be left out, with consequent increase in production.

In 1946 G. Loftus-Hills became Chief of the Division of Dairy Research and remained so throughout the Division's independent life. After it occupied new laboratories at Highett, Victoria, in 1955, the Division began to apply the principles of chemical engineering processes to dairy manufacture. One of the most challenging projects of the group was to introduce mechanisation into the age-old art of cheesemaking. A team succeeded, contrary to most predictions, in mechanising all steps in the process of cheddar cheesemaking and, by this advance and others, wrought a transformation in the Australian cheese industry[8]. Similar success was achieved in the development of a continuous process for the manufacture of casein and subsequently for the recovery of the 'coprecipitate', the total proteins of milk. Efforts to make new foods from milk led to the development of special products for infants with phenylketonuria and with lactose intolerance, and to the manufacture of a high protein milk biscuit that has found acceptance in some underdeveloped countries. The Division of Dairy Research organised the first flavour chemistry group working in Australia, in 1948, and it led the world in identifying the compounds important in the flavour of butter and in isolating compounds responsible for typical off-flavours[9]. The staff of the division has a particularly successful relationship with industry, ensuring that new developments are fully utilised.

Current interests

From a limited rescue operation for the beef and fruit trades, carried on in a single laboratory attached to the Queensland Abattoir and a few leased cold rooms in the Victorian Government's Cool Stores, CSIRO's interests in food research have developed to embrace nearly all aspects except sugar and margarine. Some recent investigations illustrate the range and quality of current work.

The mechanism of cold injury in plants at temperatures above 0 °C has long been obscure. Work proceeding in the Division of Food Research has shown that the temperature at which a phase change in the cell membranes occurs is indicative of the sensitivity of a plant to chilling injury. Spin labelling techniques have proved to be the best detectors of phase changes[10].

Sophisticated equipment is being used in broadly-based studies of the chemistry of food flavours. Among current achievements have been the identification of the components responsible for the characteristic flavours of peas and passionfruit[11].

Considerable progress has been made in removing limonin, the bitter principle of orange juice, by adsorption on cellulose acetate gel beads and by enzymatic degradation[12].

Valuable additions are being made to knowledge of the sources and control of *Salmonella* infections of meat in abattoirs, and the potential hazards to the health of consumers have thereby been greatly reduced[13].

Soon after amalgamation with the Division of Dairy Research, the Division of Food Research started the huge project of studying properties of polyunsaturated ruminant foods (made polyunsaturated as a result of a feeding regime developed by the CSIRO Division of Animal Physiology), and of ensuring that the techniques were specified for processing and storing the resulting foods on a commercial scale. The success of this venture, which has made the foods available to people needing to reduce their blood cholesterol levels, would scarcely have been achieved without close collaboration between the Division's three major laboratories.

Future trends

The research programme of the Division of Food Research has hitherto been directed mainly at the solution of the major preservation and processing problems of industry. Greater emphasis is beginning to be given to the needs of consumers and to aspects of human nutrition, and these will be helped by the recent establishment of the CSIRO Division of Human Nutrition.

In this connection, much more work will be done to provide sound microbiological information on which to base realistic legal standards to protect the health of consumers and to assist the processing, storage and handling of perishable foods. Attention will probably also be given to the technical need for various food additives and to their detection in trace amounts in foods. Work on the provision of foods of specified composition to suit people with special dietary needs will be extended beyond the current interest in animal foods with high ratios of polyunsaturated to saturated fats.

Research work aimed at solving waste disposal problems so often encountered by food factories can materially improve the human environment, particularly in rural areas, and may profit the manufacturers: an expansion of this work seems likely.

The urgent needs for mechanisation of the food processing industry are being considered by the Division of Food Research, and an early start is likely on investigations aimed at greatly increased mechanisations of the processes, slaughter, dressing, cooling and cutting up, involved in the conversion of animals into meat.

Increasing emphasis will be given to new sources of proteins for human foods. In addition to studies on the separation and utilisation of blood and whey proteins, these investigations are extending to proteins from seeds such as sweet lupins, which are well adapted to yield profitable crops in a wide range of Australian agricultural conditions.

It is most unlikely that these changes will deflect the Division of Food Research from its continuing purpose in providing essential background information on the physical, chemical and biological properties of foods.

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Minerals research

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CSIRO minerals research has come a long way since its beginning in 1940. I. E. Newnham takes a look at the progress made in that time under the guidance of several strong personalities, and outlines some of the planned future directions.

THE first Executive Committee of the Council for Scientific and Industrial Research (CSIR), later to become the Commonwealth Scientific and Industrial Research Organisation (CSIRO), showed no enthusiasm towards minerals research. Even in its eighth year it spent only £410—of a total budget of £81,000—on the partial support of a mineralogist sponsored by mining companies. Not until its Chief Executive Officer realised the need for a scientific approach to finding new gold-bearing deposits[1] did the council persuade the Treasury to ease the purse strings, and then for four years, CSIR spent £3,000 a year on "gold-mining"; finally, in 1941, it committed itself to a broader programme of minerals research.

It is debateable whether the Council realised the full significance of the step it took when, in 1940, it formed the Division of Industrial Chemistry under a chief (Dr I. W. Wark) who was determined to provide work for the minerals industry. Wark's interpretation of "non-metallic minerals and their utilisation (including ceramics and cement)"—the only Cabinet-approved area of mineral investigation for the new division—was wide and generous. His first staff appointments included Lewis Lewis, a metallurgist, as Divisional Secretary, and R. G. Thomas as Head of a Minerals (later called Minerals Utilisation) Section; together they introduced a comprehensive programme of minerals research. The influence of Wark and Lewis on minerals research policy in CSIR and CSIRO remained unbroken, though not completely unchallenged, from 1940 to 1975.

Early projects and their development

Mineral sands. The first major investigation by the Minerals Utilisation Section concerned the exploitation of the heavy minerals in the beach sands of northern New South Wales. Thomas introduced the subject in his 1941 inaugural staff talk with a characteristic sample of misquoted whimsy:

> "The Walrus and the Carpenter were walking hand in hand;
> They wept like anything to see such quantities of sand.
> 'If this were sent to USA', they said, 'it would be grand.'
> 'If seven maids with seven mops swept it for half a year,
> Do you suppose,' the Walrus said, 'that they could get it clear?'
> 'I doubt it', said the Carpenter, 'we'd better treat it here!' "

"Treating it here" was a major goal of the section.

In an attempt to establish an Australian industry based on the rutile and zircon of Byron Bay, Thomas encouraged research on the chemistry of titanium and zirconium. As a wartime exercise the Division built a small pilot plant for making titanium tetrachloride from rutile and produced titanium metal for high power vacuum tubes. It subsequently initiated a full-scale study of the esters of orthotitanic acid[2]. The discovery that polymerised butyl titanate was an excellent vehicle for heat-resistant pigments resulted in the overseas manufacture and marketing of a new line of paints. No Australian production ensued, however. Nor did it from the development of a process for separating hafnium and zirconium, based on the differential reduction of their chlorides[3]; although in that case the payment of a substantial patent licence fee by an American company mitigated the disappointment.

Twenty years were to pass before Wark's Division was able to assist the mineral sands industry in the development of the ilmenite upgrading process now used in Australia's first commercial plant. Although not foreseen by Thomas, recent advances in this industrial technology[4] stem from the work of A. D. Wadsley, who joined the Division in 1943 and attained a world reputation in chemical crystallography before his untimely death in 1969.

Uranium. In 1942 a visit from Sir Henry Tizard, and discussions with Professor Oliphant, alerted Thomas to the need for information concerning Australia's uranium resources. He enthusiastically set out to develop a suitable leaching and recovery process for treating the low grade deposits of Mount Painter in South Australia. Characteristically, his enthusiasm did not extend to the interaction with government departments which the project required; in 1944 he complained to Wark about "the cumbrous and ineffective machinery which characterises interdepartmental liaison in mineral investigation work".

The machinery ran more smoothly when the Premier of South Australia became enthusiastic about the extraction of uranium from the Radium Hill deposit in his state. CSIRO worked closely with the South Australian Government and with the Combined UK–USA Development Agency to perfect a process involving concentration by flotation, acid treatment of the concentrate, and selective ion exchanges of the resulting liquor. An industrial plant, designed and brought into operation mainly by the CSIRO group, was commissioned at Port Pirie in 1954.

Cement. From the outset, Wark sought cooperation between his division and Australian industry. In 1942 he set up a Cement Section under Dr A. R. Alderman to conduct the division's first sponsored research project. The provision of an annual subsidy of £1,500 by the Cement Manufacturers' Association of Australia marked the commencement of a 32-year collaborative study into the manufacture and properties of cements and the preparation of concretes. The major sponsor of the work, which spread into three other divisions, was the Cement and Concrete Association of Australia; it contributed over $A200,000.

The first success was the recognition of the deleterious effects which occur in cements when active amorphous silica in aggregate particles interacts with the alkali components[5]. The results of this research kept Australia free from major failures such as those which troubled the American cement and concrete industry. Other projects included the use of suitable Australian pozzolanas for the construction of dam walls; the identification of the aggregate rock which had been responsible for cracking and floor slab deformation in the Sydney area; and the formulation of specifications for the correct use of additives to concrete, either to reduce their water content or to increase their fluidity.

Coal. Although Wark established close contact with leading fuel experts from Victoria in 1944, and Lewis promoted the calling of a conference to consider the technical problems of coal use, their initial efforts to launch coal research in the Division of Industrial Chemistry were rebuffed by the Executive Committe. Wark, however, subsequently set up a major project on coal gasification, which culminated in the commissioning of a two-stage pilot reactor for producing high-Btu gas from brown coal[6].

Meanwhile, the council established a Coal Survey (later Coal Research) Section in Sydney. H. R. Brown, first as Officer-in-Charge and later as Chief of Division, pioneered CSIRO's work on such subjects as the petrography, carbonisation, and inorganic constituents of coal. Studies that elucidated the mechanism by which spheres of 'mesophase' grow during the conversion of vitrinite into coke[7] attracted international collaboration, and detailed investigation of the inorganic constituents of coal from more than 600 locations in Australia helped to solve problems of boiler-fouling and fly-ash emission.

In 1959 the amount of talent which Wark had assembled reached, so to speak, its critical mass, and one of six fission products, the Division of Mineral Chemistry, merged with the Division of Coal Research in 1967, two years after Wark's retirement. A new member of the enlarged division tracked down the cause of, and provided the remedy for, the inefficient performance of electrostatic precipitators[8]. Coal chemists and petrologists were encouraged to contribute to the techniques of prospecting for oil, gas and minerals in Australia. This group's unorthodox concepts of the genesis of oil and gas from the remains of land plants, in deeply buried sediments subjected to suitable thermal regimes, have proved significant in the understanding of Australian hydrocarbon resources.

Atomic absorption spectrophotometry. A 31-year-old Manchester physicist, Alan Walsh, recruited to the Division of Industrial Chemistry, in 1946, was to become the most spectacularly successful of the many talented scientists who commenced their Australian research careers at 'Fishermen's Bend'. At the time of Walsh's appointment the Chemical Physics Section had acquired a spectrophotometer for molecular absorption spectroscopy, and equipment for atomic emission spectroscopy. Six years later Walsh found himself wondering why molecular spectra were usually obtained by absorption techniques and atomic spectra by emission: his subsequent ideas, and the crucial experiments which followed[9], started a worldwide innovation. The technique—atomic absorption spectrophotometry—which Walsh and his coworkers evolved in the following years, has found application in many fields[10]. It ranks as CSIRO's major contribution to the minerals industry, and it is noteworthy that Australian mining companies assisted its early development.

Current research

THE establishment of new mineral-processing industries is a laudable, but restricted, objective of the CSIRO minerals research programme. The international availability of know-how, and the widespread technological base of the industry, ensure that in times of normal economic development, individual companies need no encouragement from CSIRO to introduce new mineral-processing industries to Australia. It is true that CSIRO has attempted on several occasions to fill an apparent gap in the range of domestic products by providing novel technology, a recent example being a project for producing electrode carbon by cracking natural gas on the surface of selected brown coal chars. But CSIRO's main role in establishing new areas of production is that of a complementary partner, stimulating and encouraging industry by widespread research. This is not to argue that there is no call for innovatory minerals research in CSIRO—indeed, such research is essential in achieving greater technical efficiency for established segments of the minerals industry, and the development of up-to-date investigatory techniques.

CSIRO involvement in these latter areas themselves has been questioned on political grounds, but it has resulted in a close, fruitful relationship with industry[11]. Current projects include iron ore mining and comminution, the production of iron oxide pellets, the development of International Standards for analytical procedures, the response of flotation plants to changed feed characteristics, the smelting of low grade tin concentrates, and the disposal of coal washery rejects and metal refining by crystallisation and reflux.

Modern instrumental technology is being used to solve problems in areas such as borehole logging, the remote sensing of minerals, and on-line analysis. Techniques that are subject to detailed study include energy dispersive X-ray fluorescence analysis, transient electromagnetics, quantitative X-ray diffraction analysis, fluid inclusions, microanalysis and isotope geochemistry.

CSIRO is also concerned with the creation (in cooperation with State and Federal Government Departments and Agencies) of a technical base which evaluates resources and anticipates the needs of the nation. Though excellent in concept, this aim can be difficult to achieve—consider the misunderstandings which attended recent attempts to promote liaison between CSIRO and the former Department of Minerals and Energy. Fortunately, there has emerged a Joint Department—CSIRO Research Advisory Committee—which will be concerned with the formulation and modification of future mineral research programmes. In addition to its long-standing associations with state mining departments, electricity authorities and geological surveys, CSIRO is currently forging close links between its mineral divisions and various environmental groups at both State and Federal level.

During the past decade the Executive has encouraged divisions to adopt a management system which helps to define the intent and output of CSIRO's research programmes[12]. This has resulted in a method of annual reporting which quantitatively identifies the movement of research staff into new areas of investigation. Figure 1 shows the changes that have occurred in the Minerals Research Laboratories during the past five years, as well as those projected for 1976–77. Of the 194 members of the professional staff employed in 1971, 59 have moved into new programmes falling under the categories of 'environment' and 'energy' (Fig. 1). This movement has not occasioned any major disruption of the structure of individual divisions.

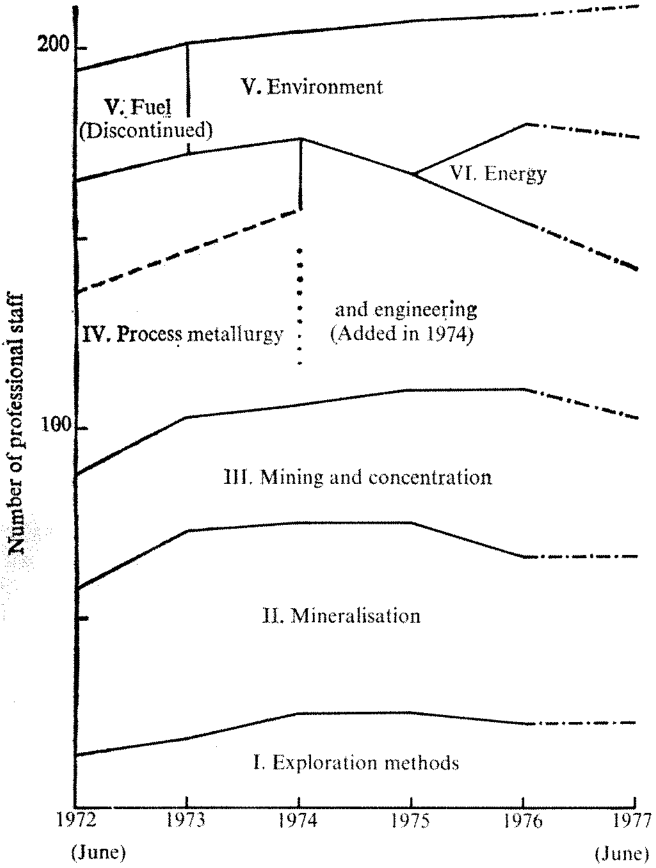

Fig. 1 Professional manpower resources allotted to the programmes of CSIRO Minerals Research Laboratories, 1971–76.

Inorganic chemists do not become geophysicists, or vice versa, but both agree to apply their combined skills to the solution of problems in areas with which they had no previous academic association. Many of these changes originate at the level of the research scientist.

If there is a budding Walsh among the new generation of CSIRO scientists, his Chief can use this flexibility to allow his work to expand in unexpected directions without having to justify such action by extravagant prediction or premature speculation. It is a Chief's responsibility to foster exceptional talent in ways that his own judgment best dictates.

Future trends

A major goal in the next decade will be to cultivate the greatest possible skill in those areas of science that satisfy or anticipate industrial and community needs. No matter how practical these needs may be, efforts to meet them at times lead research workers into realms of academic theory; such movements are deemed essential to CSIRO minerals research.

Several activities in the six major programmes of the Minerals Research Laboratories are, during the next decade, likely to require additional effort by the constituent divisions of Chemical Engineering, Mineral Chemistry, Mineral Physics, Mineralogy and Process Technology.

Exploration methods. There will be a continuing emphasis on improving the technology of exploration. A thick and often saline overburden renders exploration difficult in many parts of Australia. Basic research into electrical geophysics, rock magnetism, the interpretation of aerial photography, and several forms of geochemistry will be linked with the specialised field knowledge and experience of the exploration industry, state geological surveys and the Bureau of Mineral Resources.

Mineralisation. A clear understanding of the genetic environment of an economic mineral deposit, if it can be reliably associated with chemical and/or physical features of the rocks, may form the basis of a valuable exploration technique. Because Australian geology shows many similarities to that in parts of India and southern Africa, there is a possibility of finding Australian equivalents to the economic deposits of these other countries. A prime target is the discovery in the north-west of the continent of gold–uranium deposits analogous to the 'banket' of the Witwatersrand. The art of prospecting still awaits the solid reinforcement of academic theories on Precambrian environments; CSIRO can help to build these theories and develop them into practical advice for the exploration industry.

Mining and concentration. The development of new mining methods—particularly the application of computer techniques to the solution of underground problems—must be studied in Australia. The extraction of coal from thick seams at depth requires urgent investigation. There will be an increasing emphasis on process control, particularly as new and better on-line sensors are developed, and computers become smaller and cheaper. A system will be developed for extracting on-line positional and compositional information from particles whose image is created by the beam of a scanning electron microscope.

Process metallurgy and engineering. This programme is concerned with the transformation of minerals into materials of increased value and of greater marketability. In the important field of pellet formation there will be some need for research on the roles of solid state diffusion, slag formation and migration, and bonding between the liquid and solid phases. The gradual depletion of existing reserves will necessitate the development of hydrometallurgical and pyrometallurgical methods for treating low grade complex ores. There will be an increased need to study high intensity pyrometallurgical reactors.

Environment. Increased research effort will be devoted to understanding the influence of industrial and chemical processes on the biosphere and, where possible, to ameliorating any undesirable effects. In particular there is a need for field studies supported by laboratory work on photochemical smog, urban haze, pollution by sulphur dioxide and nitrogen oxide, and the effect of heavy metals on aquatic life.

Energy. There will be continued emphasis on longer term projects related to oil and gas exploration, the efficient mining of coal, the possible conversion of coals into liquid fuels, alternative energy sources and energy storage. Insufficient knowledge of the nature and location of source materials, and of the conditions controlling hydrocarbon generation and accumulation, is available to guide exploration in off-shore areas of the North-west Shelf and Bass Strait. CSIRO will continue to provide data and ideas to support the companies exploring these fields and certain inland basins. The complicated and variable constitution of coal makes it essential that fundamental work should accompany practical studies of the most suitable process, or combination of processes, for converting coal into oil in Australia. There is an urgent need to understand the chemical and physical properties of those fractions of Australian coal which are enriched in particular macerals, and to assess the influence of heating rate on both the initial and subsequent processes of depolymerisation which affect the yields of tar and gas.

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Wool structure and biosynthesis

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Originally set up to assist the textile processing and manufacturing industries, the CSIRO Wool Research Laboratories have contributed significantly to fundamental knowledge of the chemistry and biology of wool.

> "Captain Macarthur considers it his duty respectfully to represent to His Majesty's Ministers, that he has found, from the experience of many years, the climate of New South Wales is peculiarly adapted to the increase of fine-woolled sheep . . . Captain Macarthur being persuaded that the propagation of these animals would be of the utmost consequence to this country, procured in 1797 three rams and five ewes, and he has since had the satisfaction to see them rapidly increase, their fleeces augment in weight, and the wool very visibly augment in quality."

THUS wrote Captain John Macarthur to Lord Hobart in 1803[1]. His prophecy was amply fulfilled—the sheep population of Australia currently exceeds 150 million and the annual production of wool is around 770 × 10^6 kg with a value of £620 million. Research for the benefit of the wool industry is financed through grants from a Wool Research Trust Fund which is replenished partly by a levy on the sale of wool and partly by direct contributions from the Australian Government. The fund provides grants for both promotion and research and the bulk of the research is carried out by the Commonwealth Scientific and Industrial Research Organisation (CSIRO). The grant to CSIRO for wool research during the financial year 1975–76 was approximately £8 million.

During the first two decades after the formation of the Council for Scientific and Industrial Research (CSIR), wool research was concerned primarily with production and the main benefits to the grower were in terms of increased production and improved quality. Towards the conclusion of the Second World War, production of both synthetic and regenerated man-made fibres increased and this was seen as a threat to wool's future as a textile fibre. Accordingly, CSIR in 1945 invited four leading textile scientists to advise on the establishment of a facility in Australia for wool textile research, with the aim of assisting the wool processing and manufacturing industries both in Australia and overseas. One of the advisers was J. B. Speakman who, at that time, was Professor of Textile Technology in the University of Leeds. He recommended the establishment of a protein research laboratory devoted to the study of the chemical and physical structure of wool and its chemical reactivity, a textile engineering research laboratory, a textile technological research laboratory and a wool grease research laboratory. In 1948 the decision to undertake wool textile research in Australia was implemented with the establishment of three laboratories which today constitute the CSIRO Wool Research Laboratories; the Division of Protein Chemistry situated at Melbourne, the Division of Textile Physics situated at Ryde in New South Wales, and the Division of Textile Industry situated at Geelong in Victoria, which handles studies of wool grease as part of its programme. Australia was not alone in seeing the need for wool-growing countries to be concerned with the end use of the product as well as its growth, and since 1964 wool textile research in South Africa, New Zealand and Australia has been coordinated through the Research and Development Committee of the Board of the International Wool Secretariat.

Wool has a number of characteristics which are highly desirable in a textile fibre. It can readily be dyed to a wide range of colours and shades, it can be set into desired shapes and yet shows good wrinkle resistance and recovery, and it can be compacted when required by controlled felting. It is also a very safe fibre compared with cotton and synthetics since it tends to char rather than burn and when ignited burns only slowly. Wool garments have outstanding heat insulation properties and yet allow free movement of moisture and air, thus making them comfortable to wear. On the other hand, untreated wool is susceptible to attack by larvae of the clothes moth and is damaged by prolonged exposure to sunlight, and untreated wool garments shrink and lose their set when washed or exposed to warm, humid conditions. The aim of studying wool structure and biology has been, first, to provide background knowledge to serve as a basis for understanding wool's properties, both good and bad, and second, to suggest ways in which these properties might be modified to advantage. At first sight it might be thought that studies of the physical and chemical nature of wool alone might be sufficient. But wool is a highly variable product and the rate of wool production, the distribution of fibre diameters, the crimp or curl, the mechanical properties and colour are all affected by the health, nutritional state and environment of the sheep and so in turn are the processing characteristics of the wool. Studies of the biology of wool growth thus form an essential adjunct to structural studies in providing background knowledge for the benefit of the wool textile technologist, quite apart from their intrinsic value in wool production.

The foundations of present knowledge of the molecular structure, mechanical properties and chemical reactivity of wool were laid in the early 1930s by W. T. Astbury and H. J. Woods and by J. B. Speakman, all of whom worked at the University of Leeds. Wool is naturally insoluble due to the presence of covalent disulphide linkages between the molecular chains, but by 1935 D. R. Goddard and L. Michaelis working at the RockefellerFoundation in New York had isolated relatively intact proteins from wool solubilised by reduction. The remarkable complexity of the wool fibre first became apparent after the low-angle, X-ray diffraction studies of I. MacArthur at Leeds, and the early electron microscope studies at the CSIRO Division of Chemical Physics in Melbourne. The formation of the CSIRO Wool Research Laboratories provided, for the first time, facilities for an integrated programme of research into the mechanical properties, molecular structure and chemical reactivity of wool. In addition, it provided an opportunity for research results to be exploited for the benefit of the wool industry within the one group of laboratories.

It is not possible here to review adequately the extensive programme of research on wool biology and wool textiles that have been carried out in the CSIRO laboratories. As indicated by the title, this article is concerned primarily with wool structure and biosynthesis and the following sections give a brief account of the current state of knowledge on these topics.

Biosynthesis

In 1927, shortly after the formation of CSIR, a laboratory was established in Adelaide for the study of the influence of nutrition on the biochemistry and physiology of the sheep. The basic knowledge acquired in this study revealed that animal health and wool production in a number of areas were adversely affected by deficiencies of copper, cobalt and zinc in the soils. Spectacular improvements resulted when traces of the deficient metals were supplied either as a dietary supplement or in a superphosphate top-dressing to the soil. At present research which bears either directly or indirectly on wool biosynthesis is being carried out in many CSIRO divisions. Investigations of soil, pastures, diseases and parasites, genetics, flock husbandry and management, and physiology of sheep reproduction, nutrition and wool growth have all contributed to more efficient wool production.

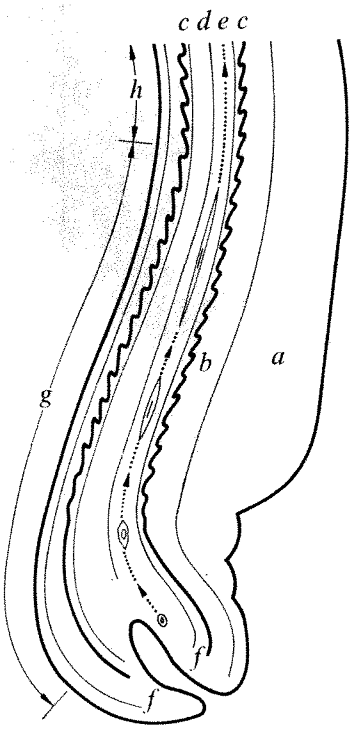
In most mammals the pelage consists of an outer layer of coarse, heavily medullated fibres and an inner layer of much finer fibres. In the case of fine-woolled sheep the coarse outer layer is absent. Another difference is that most mammals have a growth-replacement cycle but in fine-woolled sheep the growth is effectively continuous.

Wool is produced in follicles formed by invagination of the epidermis and at the base, or bulb, of the follicle there is an invagination of the surrounding connective tissue termed the papilla (Fig. 1). In the region surrounding the papilla proliferation of cells destined to form the fibre and the inner root sheath occur. The central stream of cells elongate during their passage through the follicle and form the cortex of the wool fibre. A second stream of cells, surrounding the first, become flattened and form the cuticle of the fibre. No cell divisions are observed in either of these streams above the bulb region. Additional cell streams destined to become the inner and outer root sheaths envelop the fibre cells.

Wool proteins, termed keratins, are synthesised and laid down intracellularly as the fibre cells progress up the follicle. The cortical cells fill with fine filaments which are embedded in a cystine-rich matrix. The available evidence suggests that the synthesis of filament and matrix proteins takes place concurrently but the relative rates of production do not seem to be equal, with the relative rate of production of the matrix proteins increasing sharply during the latter stages of biosynthesis.

Initially the keratin proteins are laid down in the thiol form but as the fibre cells mature the cysteinyl residues are oxidised in pairs in a copper-catalysed reaction and the resulting disulphide cross linkages render the structure insoluble in the natural environment and resistant to attack by microorganisms. This process is termed keratinisation.

Fig. 1 Diagrammatic longitudinal section (foreshortened) of a fine-wool follicle. *a*, Outer root sheath; *b*, inner root sheath; *c*, fibre cuticle; *d*, paracortex; *e*, orthocortex; *f*, undifferentiated region; *g*, zone of maturation of fibre cells; *h*, fully formed fibre.



During the passage between the bulb region and the zone of keratinisation a layer of material is deposited between the cells and the cell membranes are modified. In the final fibre the resulting complex effectively binds the keratin in the individual cells into a mechanically continuous material. The combined effects of disulphide cross linking and deposition of the cell membrane complex give wool a high temperature stability and the effect of temperature on the initial stretching modulus of wool in water is only slight up to 100 °C.

Breed and nutrition have an important influence on the processing and textile properties of wool and research has been undertaken in the Division of Textile Industry to compare the processing properties of a range of wools and also the fabrics produced from them. Fibre diameter was found to be the most important single factor affecting the so-called handle or softness of the fabric, with the finest wool giving the softest fabric. For wools of a given diameter range the fabric properties were generally insensitive to variations between wools but the processing properties varied considerably. In addition to direct practical tests, basic studies of the biosynthesis of wool have been carried out in the Division of Animal Physiology and in the Division of Protein Chemistry with the aim of understanding the effects of breed and nutrition on fibre production, structure and properties.

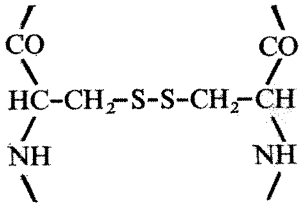
Rate of wool growth depends on the genetic background of the animal, its nutritional status and a number of physiological factors. Experiments carried out at the Division of Animal Physiology have established that both wool production and the sulphur content of the wool are influenced by the availability of sulphur-containing amino acids in the diet. This was controlled by intra-abomasal feeding and the content of high sulphur proteins in the wool varied within the range 18–34%. A number of dietary treatments, for example feeding with cereal proteins, have been found to suppress production of certain glycine-rich matrix proteins but so far none has been found to increase their proportion.

In animals maintained on a copper-deficient diet the copper-catalysed cross-linking stage in keratin synthesis is defective and a grossly abnormal fibre is produced. A similar situation, associated with genetic defects, occurs in both mice and men.

The product of the wool follicle is very unusual in that both the proportions of the individual proteins and the cross linkages between them can be manipulated within wide limits by dietary means. Potentially this provides a method of varying the properties of wool for particular end uses. A wide variety of agents are known which will suppress wool growth. Most are toxic, but in recent years studies have been carried out in the Division of Animal Production to test the feasibility of "chemical shearing" in which the animal is either injected with or fed a substance which causes a temporary interruption of wool growth and subsequent shedding of the fleece.

Wool proteins

Wool is almost entirely composed of cystine-containing proteins termed keratins and, as mentioned earlier, the natural insolubility of wool is attributed to the presence of interchain disulphide linkages formed by post-synthetic oxidation of pairs of cysteinyl residues (I).



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A major programme of the Division of Protein Chemistry has been concerned with the solubilisation of wool and the prepara-

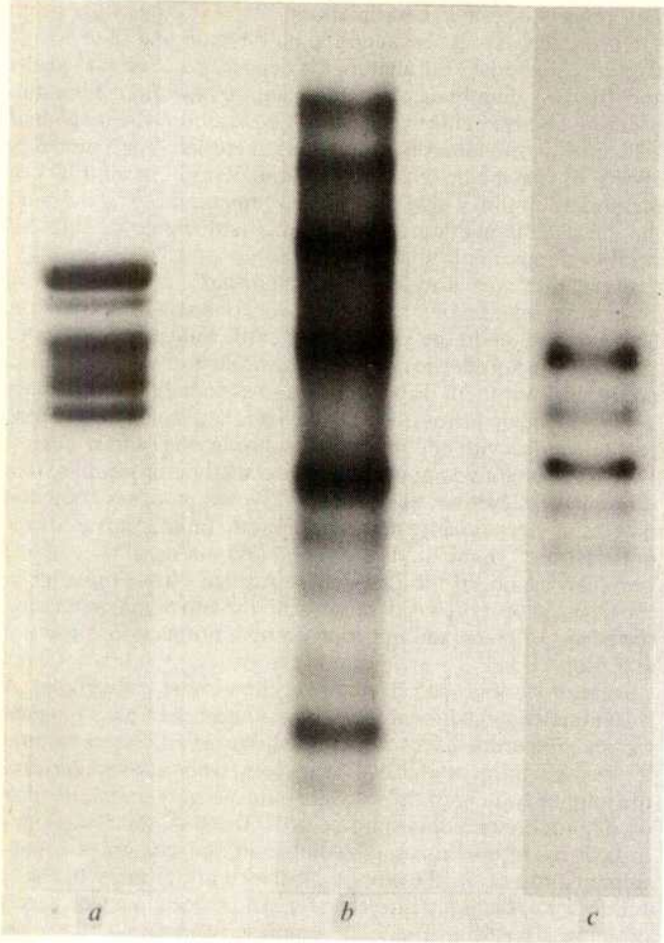


Fig. 2 Electrophoretic patterns of the three groups of wool proteins. *a*, Low sulphur group run in sodium dodecyl sulphate–polyacrylamide gel, *p*H 7.0. *b*, High sulphur group run in polyacrylamide gel in urea buffer, *p*H 2.6. *c*, Glycine-rich group run on cellulose acetate in a urea buffer, *p*H 8.9. (Division of Protein Chemistry.)

tion of derivatives suitable for the analysis of protein composition, and the purification and characterisation of individual protein components. Care must be taken to prevent peptide bond hydrolysis during the procedures used for breaking disulphide bonds and the subsequent extraction of proteins. Much early work was concerned with the evaluation of different methods for breaking disulphide bonds, and procedures were developed using peracetic and performic acids, tributyl phosphine, oxidative sulphitolysis, sodium thioglycollate and mercaptoethanol. In more recent work the latter two reagents have generally been used and the extracted protein subsequently treated with sodium iodoacetate to give the *S*-carboxymethyl derivatives. This prevents reformation of the disulphide linkages and also improves solubility.

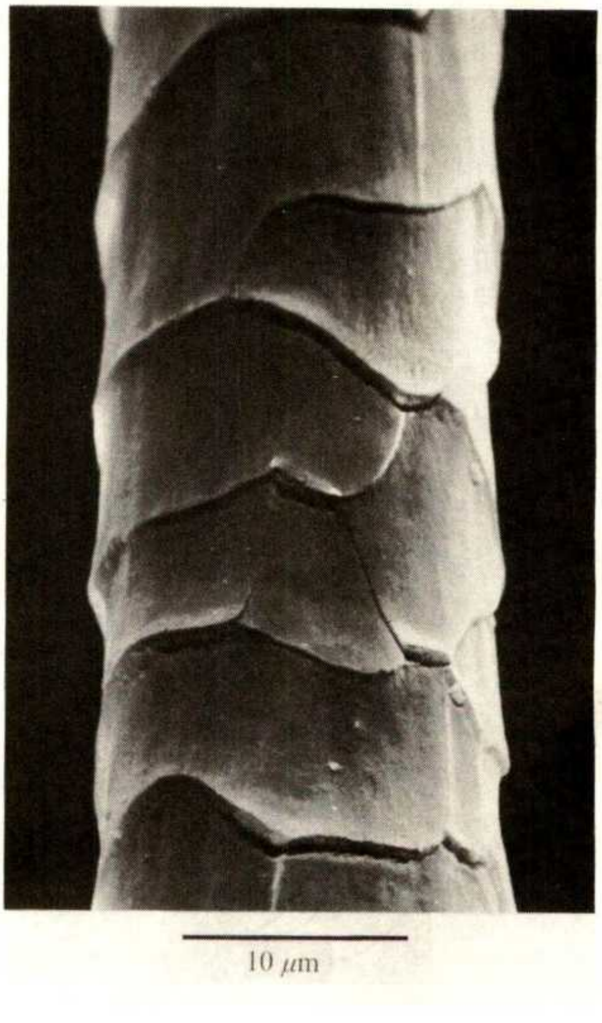
Three main groups of wool proteins have been identified and isolated. Proteins in the low sulphur group (Fig. 2*a*) have fewer cysteinyl residues per cent than whole wool and have molecular weights in the range 40,000–58,000. In solution the *S*-carboxymethyl derivatives of these proteins have an α-helix content of about 50% and helix-rich chain segments about 100 residues long can be isolated after partial hydrolysis with proteolytic enzymes. Two such segments have been sequenced and the distribution of non-polar residues was found to have the 7-residue periodicity predicted by F. H. C. Crick for the coiled-coil conformation. Oriented films of the fragments exhibit the characteristic X-ray diffraction pattern of the coiled-coil rope structure.

Proteins in the high sulphur group (Fig. 2*b*) have more cysteinyl residues per cent than whole wool and have molecular weights principally in the range 10,000–28,000. In solution the *S*-carboxymethyl derivatives of these proteins do not appear to contain any α helix. The total number of high sulphur proteins in a wool fibre probably exceeds 100 and various subgroups have been recognised. Proteins from the low and intermediate molecular weight subgroups have been sequenced by South African wool scientists while several proteins in the upper molecular weight range have been sequenced in the Division of Protein Chemistry. These latter sequences exhibit a slightly imperfect decapeptide repeat, the structural significance of which is unknown.

Proteins in the third group (Fig. 2*c*) are characterised by a high content of glycyl residues (up to 40%) and a higher than average content of aromatic residues (up to 34%). The number of glycine-rich proteins in a wool fibre probably exceeds 50 and two subgroups have been recognised which differ in their contents of cysteinyl residues and phenylalanyl residues. The molecular weights of the glycine-rich proteins are generally below 10,000 and in solution the *S*-carboxymethyl derivatives do not seem to contain any α helix. So far only one glycine-rich protein has been sequenced and the only evidence for regularity is in the occurrence of segments in which glycyl residues alternate with non-glycyl residues. A similar alternation is found in silk fibroin from the silk moth *Bombyx mori* and is there associated with a special mode of packing of β-pleated sheets. The significance of this alternation in the case of wool proteins is unknown.

Although much progress has been made in the isolation and characterisation of wool proteins there is virtually no evidence concerning specific interactions between proteins in different groups. In such a highly organised structure as wool it is likely

Fig. 3 Scanning electron micrograph of the surface of a fine wool fibre showing the projecting edges of the cuticle cells. The direction of the tip is towards the bottom of the micrograph. (Division of Textile Industry.)



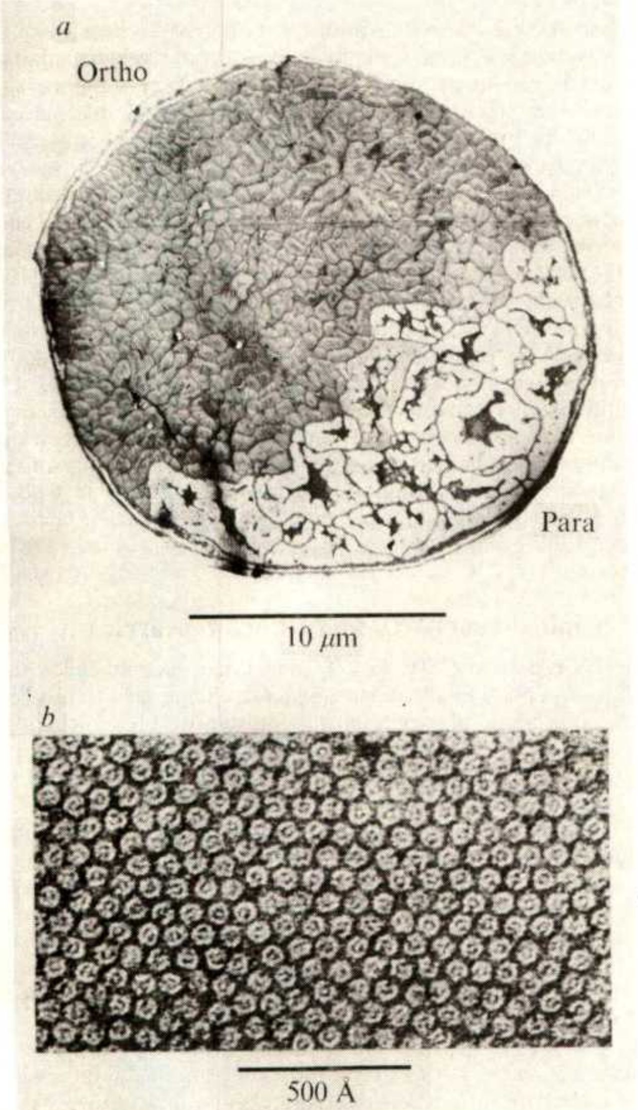


Fig. 4 Electron micrographs of cross sections of fine wool. *a*, Low power micrograph showing the cuticle, and the orthocortex and paracortex. *b*, High power micrograph showing the filament–matrix texture. (Division of Protein Chemistry.)

that aggregates of precise stoichiometry are present and one aim of present research is to determine their nature.

Wool structure

A wool fibre from a Merino sheep is typically 20–30 μm in diameter and the exterior portion, amounting to about 10% of the weight, consists of a layer of flattened cells which overlap to form a continuous tube termed the cuticle (Fig. 3). The projecting edges of the flattened cells are believed to play a part in anchoring the fibre in the follicle and in excluding foreign matter from the fleece. In addition they give wool its unique property of felting. This property is valuable in that the density of a fabric can be increased by mechanical means but the same property leads to the familiar felting shrinkage when untreated woollen garments are washed. Several new processes for preventing felting shrinkage have been developed in the Division of Textile Industry and improvements to the dry chlorination process have been made in the Division of Protein Chemistry. All involve masking or modifying the projecting cell edges and in some instances fibres are also chemically "spot welded" to prevent the irreversible relative movement between fibres which leads to shrinkage. The membranes around the cuticle cells are consolidated during biosynthesis to form an outer envelope around the fibre which is termed the epicuticle. Although very thin, this layer appears to influence the textile properties since dyeing proceeds more rapidly when the epicuticle has been damaged mechanically or chemically. The chemical composition of the cuticle differs from that of the remainder of the fibre, and cuticle cells do not seem to contain any low sulphur proteins. The bulk of the cuticle is composed of high sulphur proteins and work is in progress to compare the high sulphur proteins of the cuticle with those present in the rest of the fibre.

In coarse animal hairs the main body of the fibre, enclosed by the cuticle, consists of a dense annular cortex and a central vacuolated medulla. In fine wool the medulla is absent and so the cortex occupies the entire volume enclosed by the cuticle. The cortical cells are highly elongated and in fine, highly crimped wools, which are the main product of the Australian sheep industry, the cells are of two distinct types and are grouped together as shown in Fig. 4*a* to form a segmented filament. The topographical relationship between the segments is such that one, termed the orthocortex, is always on the outside of the crimp wave while the other, termed the paracortex, always remains on the inside of the crimp wave. The chemical and physical properties of the orthocortex and the paracortex are quite different and wool scientists in numerous laboratories throughout the world have sought to devise methods of isolating and characterising the two types of cortical cell. No definitive answers have yet been obtained but there is some evidence that the differences in properties are due in part to differences in protein composition, with the paracortex containing more high sulphur protein than average and the orthocortex containing more glycine-rich protein than average.

Electron microscope studies reveal that the cortical cells have a filament–matrix texture (Fig. 4*b*) and characteristic differences between the filament packing in the orthocortex and in the paracortex have been noted by both Australian and other wool scientists. Although much has been written about the orthocortex–paracortex structure it must be remarked that a clear understanding of its relation to crimp has not so far been obtained. In view of the importance attached to crimp by wool buyers this is clearly a field in which a sustained research programme is warranted.

Much circumstantial evidence has accumulated that the filaments in the cortex, termed microfibrils, are composed of low sulphur proteins and that the matrix is composed of high sulphur proteins. Recently it has proved possible to isolate microfibrils in a purified state, and to demonstrate directly that they contain low sulphur proteins. The glycine-rich proteins are only a minor component of most wools and their location was until recently unknown. After the discovery that some epidermal appendages such as quills contain high proportions of glycine-rich proteins it was possible to show by means of a combined quantitative extraction and low-angle X-ray diffraction study that the bulk of the glycine-rich proteins reside in the matrix.

Much use has been made in both the Division of Protein Chemistry and the Division of Textile Physics of X-ray diffraction in the study of the structure and mechanical properties of wool. The wide-angle diffraction pattern of wool has been extensively studied in the Division of Protein Chemistry and shown to be well accounted for by supposing that the α-helices in the low sulphur proteins are twisted together to form coiled-coil ropes. The low-angle equatorial diffraction pattern is determined by the axial projection of the microfibril and also by the packing arrangement of the microfibrils, and an understanding of this pattern has enabled estimates to be made of microfibril diameter and also the mean distance between nearest neighbours. Comparison of the patterns for dried and hydrated wool revealed that most of the water taken up by the wool fibre is accommodated in the matrix. This suggests chemical modification of the matrix as a likely method of influencing the many properties of wool that are humidity dependent.

Great interest attaches to the determination of the way in which the low sulphur proteins are assembled to form the microfibril. This information is contained in the X-ray diffraction pattern of wool but interpretation is hampered both by the very large axial identity period of the microfibril and by the lack of information on the stoichiometry of the low sulphur protein molecules. The most recent studies suggest that the microfibril has a helical symmetry with a repeating group of low sulphur proteins disposed at axial intervals of 67 Å on a helix of pitch 220 Å. The molecular weight of the repeating unit is estimated to be about 230,000, corresponding to four to six molecules. As with many helical structures there is evidence of periodic axial distortion.

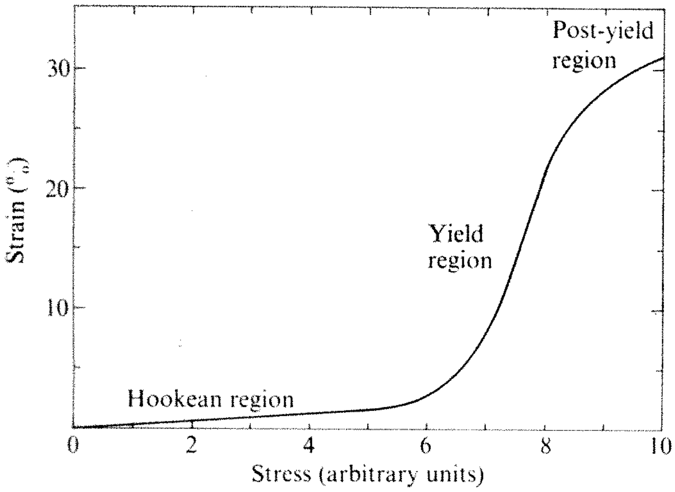


Fig. 5 Stress–strain curve for the extension of a single hair in water. (Division of Textile Physics.)

Mechanical properties

Composite filament–matrix textures are common in nature and used widely in man-made structures. In general the filaments are composed of a material with a high modulus of elasticity and the matrix of a material with a low modulus of elasticity. The special advantage of such a texture is the behaviour under stress. The matrix, which is easily deformed, distributes the stress evenly over the filaments thus preventing the propagation of cracks from local imperfections or points of rupture. Studies carried out in the Division of Textile Physics highlight the added subtlety in the case of wool that the elastic modulus of the matrix is highly dependent on water content. In a dry fibre the modulus approaches that of the filaments, but in a fully hydrated fibre the matrix behaves like a highly swollen gel. This observation provides the key to understanding many important features of the processing properties and performance of wool as a textile fibre.

A typical stress–strain curve for a naturally straight keratin fibre (hair) is shown in Fig. 5. In the case of wool there is an initial region of high slope in the stress–strain curve associated with decrimping of the fibre. Up to 2% strain, the relationship between stress and strain is linear (Fig. 5) and the strain is accommodated in the individual protein molecules. Beyond 2% strain the fibre yields readily with increasing stress but stiffens up again at about 25% strain. X-ray studies suggest that the microfibrils can only accommodate about a 2% strain before some breakdown occurs in the helical structure. Above about 2% strain there is a progressive change in the high-angle X-ray diffraction pattern with the appearance of new reflections associated with the β conformation. The transformation from the α conformation to the β conformation has been studied in some detail in the Wool Research Laboratories because of its relevance to setting. Set is defined as the strain retained after the removal of an applied stress and may be temporary or permanent. Temporary set is generally undesirable since it leads, for example, to wrinkling; permanent set is a useful property which can be used to shape clothing.

Extensive studies of the viscoelastic properties of wool and their dependence on temperature and hydration have been carried out in the Division of Textile Physics to gain an understanding of the associated textile properties. Methods of measuring fabric properties have been developed at the Division of Textile Industry and used in the development of methods for producing durable pleats and creases in woollen fabrics and for conferring wash-and-wear properties. The influence of chemical reagents on the viscoelastic properties of wool have also been extensively studied in the Wool Research Laboratories. In particular the facilitation of conformational change by a thiol–disulphide interchange reaction, first noted by R. W. Burley, has been found to be an important factor in creep and stress relaxation.

Chemical reactivity and future research

In his report to CSIR in 1945, Speakman recommended that "... first place in any scheme of research intended to promote the utilisation of wool must be given to organo-chemical studies of the reactivity of the keratin molecule" and extensive programmes of research have been carried out both in the Division of Textile Industry and in the Division of Protein Chemistry on reactivity of wool in relation to bleaching, dyeing, sunlight yellowing, removal of vegetable matter by carbonising, enzymatic hydrolysis, interactions with polymers, resistance to insect damage and flammability. In some cases the approach has been semi-empirical, in others extensive use has been made of the basic knowledge gathered on the structure of wool.

The aims of research on wool structure have been to accumulate basic knowledge concerning the molecular structure, mechanical properties and chemical reactivity of the wool fibre and to relate this to the processing and textile properties of wool. The aims of research on the biosynthesis of wool have been to obtain an understanding of the physiological and nutritional factors that influence wool growth and quality.

Much has been achieved during the past 50 years. Some research results have found immediate practical application, some have provided basic knowledge of wide applicability and some have merely served to draw attention to our ignorance. Some of the major gaps that need to be filled by future research concern the basis of the regulatory control of the biosynthesis of the keratin proteins, the arrangements of the protein molecules in the microfibrils and in the matrix, the distribution of disulphide linkages within and between the protein molecules, and the influence of structural variations on textile properties. In addition there is an urgent need for more information about the structural basis of the photochemical degradation of wool, wrinkling, setting and about the relationship between fibre and fabric properties.

When these objectives have been attained the way will be open to modify wool's properties in a predictable manner either by control of synthesis or by chemical or physical treatments.

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Fifty years of plant research

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Over the past 50 years the present Division of Plant Industry has evolved from relatively modest beginnings as the Division of Economic Botany with a brief to undertake basic research in plant sciences, to a Division whose work now touches on many aspects of crop and pasture plants, as well as the natural vegetation of Australia.

AT the time of establishment 50 years ago of the Council for Scientific and Industrial Research (CSIR), the agricultural and pastoral industries were predominant in Australia's economy. The most pressing research problems were in the field of agriculture, and these were the concern of all the first laboratories to be established. The Departments of Agriculture of the six States had been actively engaged in agricultural research for many years. Keenly aware of the need to secure their co-operation, Rivett arranged a meeting with the Directors of Agriculture, which agreed that CSIR should concentrate on problems "which are regional or national in scope, fundamental in character, and which require concentration of effort and highly specialised research for their solution". The introduction and nutrition of plants, genetics and the biology of plant diseases were cited as investigations suitable for CSIR, whereas fields such as plant breeding and the control of plant diseases were considered more appropriate for the State Departments. It was within this somewhat arbitrary framework that the Division of Economic Botany, now called Plant Industry, began its work.

Plant diseases

B. T. Dickson, a plant pathologist who came from Canada to be the first Chief of the Division (1928–50), initiated research on a wide range of plant diseases, including take-all and flag smut of wheat, blue mould of tobacco, and water blister of pineapple. Virus-free stocks of potatoes were released, with a substantial impact on yield, and the possibilities for genetic or chemical control of several diseases were explored.

The most striking early success was in the control of blue mould of tobacco, caused by the fungus *Peronospora tabacina*, which threatened to wipe out tobacco growing in Australia. H. R. Angell found that exposure of seedlings to benzol vapour provided effective control, and this practice undoubtedly saved the infant industry following its introduction in 1935. Recent research on blue mould has concentrated on the breeding of resistant varieties. Sources of resistance to the two predominant strains of the organism were located in some of the wild Australian species of *Nicotiana*, and have been transferred to cultivated tobacco, so that resistant cultivars now account for nearly all of the tobacco currently grown in Australia.

Another disease, of current concern throughout Australia, is the dying back of many species of eucalypts and other trees caused by the fungus *Phytophthora cinnamomi* and related organisms. As with blue mould, there has been a differentiation of strains by the pathogen and of resistance by the host, with important implications for their ecology and control. Such soil-borne diseases, and the complex microbial interactions involved in them, will probably require more of our attention in the future.

Apart from research on specific diseases, the Canberra plant pathologists have been active in developing the concept of phytoalexins, compounds which are produced by the host plant in response to infection and which then limit the development of the pathogen. This concept was first put forward by K. O. Müller, a Research Fellow in the Division for many years, and his colleagues subsequently isolated and identified pisatin and other phytoalexins. A polypeptide which elicits phytoalexin formation has also been identified and much is now known of the biology of the response system, but to what extent these defensive reactions can be used to control plant diseases remains to be seen.

Introduction of useful plants

The native species of *Nicotiana* have already proved of value in breeding programmes, and there is much international interest in Australian species related to cotton, soybean, sorghum and rice as sources of useful genes. The aborigines did not domesticate any plants, however, and all the crops and improved pasture plants used in Australia have come from overseas, several having been brought into the country by chance in earlier years.

Deliberate plant introduction has been a major activity of the Division since its establishment, our accessions numbering more than 70,000 out of an Australian total of about 100,000. Cereals and temperate zone pasture plants predominated in the early years, then grasses and legumes of potential value in the more tropical pastures of northern Australia, while currently the major emphasis is on crop plants. The Division has mounted 12 collecting expeditions since 1947, the first two being to South America and the Mediterranean, the most recent to the USSR.

The many introduced pasture plants were evaluated in the Division's network of field stations throughout Australia, and cooperatively with State Departments of Agriculture. More than 70 introductions have proved to be of direct commercial value as pasture plants, and many others have been used in plant breeding programmes. For example, legumes better adapted than subterranean clover to the drier and more alkaline soils of the wheat belt are now available as a result of the introduction and hybridisation of a range of medics from the Mediterranean region.

Land surveys and pasture research

Pasture improvement has always been a major concern of the Division. The native grasses were studied initially, and a survey of Australian grasslands was undertaken. One result was a sharper realisation of how little was known of the agricultural potential of northern Australia, and several field stations were established there. A series of land-use surveys was made under C. S. Christian, whose unit was eventually established as a separate Division of Land Research.

Agrostological research also grew rapidly in the 1940s under J. Griffiths Davies. New regional laboratories and field stations were set up throughout Australia. In the south, the finding of better ways to establish subterranean clover and of more effective fertiliser practices were crucial to the rapid extension of improved pastures. In the north, where the group under Davies transferred and eventually became the Division of Tropical Pastures, the introduction and improvement of a wide range of exotic grasses and legumes was a key element in their success.

More recent pastoral research at Canberra includes the breeding of improved legumes and grasses, such as cultivars of *Phalaris tuberosa* with greater seed retention and seedling vigour

The first three chiefs of the Division of Plant Industry: from the left, Sir Otto Frankel (1951–62), Dr B. T. Dickson (1928–50) and Dr J. E. Falk (1963–70).

and reduced tryptamine content. The improved plants have been assessed by measurements not only of pasture growth but also of the productivity of animals grazing on them. Computer simulation techniques are currently being used to bring together the results of many diverse grazing trials, and to explore the integration of cropping and grazing systems.

Plant nutrition and soil fertility

In their natural state most Australian soils are deficient in phosphorus, and many of the native plants are adapted to low levels of that element. Increase in phosphorus status, through the application of superphosphate to both crops and pastures, has been crucial to the building up of fertility and productivity. Australia uses about 5% of the world's phosphatic fertilisers, compared with only 0.5% of the nitrogenous fertilisers, and much attention has been given to optimising the methods of application and following the fate of the applied phosphorus.

Not all the responses to superphosphate are due to its phosphorus content. In 1949 sulphur deficiency was shown to occur in pastures on Southern Tableland soils, and since that time many sulphur-deficient soils have been identified in Australia. Their amelioration by superphosphate may, in some areas, accentuate selenium deficiency, a problem currently under investigation, as is the fractionation and cycling of sulphur compounds in soil. The Division also played a major part in the recognition and amelioration of molybdenum deficiency in Australia, and first demonstrated that appreciably more molybdenum is needed by legumes for symbiotic nitrogen fixation than for nitrate utilisation. We now know that molybdenum is a component not only of nitrate reductase but also of nitrogenase, a key enzyme in the process of nitrogen fixation. A recent twist to this older finding is that as the nitrogen level in soils has been built up by legume leys, increasing levels of molybdenum are required by pasture grasses and wheat crops to avoid injury by nitrates.

The impact of research by the Division of Plant Industry on pasture improvement, especially by better nutrition, has been subject to econometric analysis by Duncan[1], who found very high internal rates of return (25–80% per annum) on a number of projects.

Physiological and genetic basis of plant productivity

Under the second Chief, the cytogeneticist and plant breeder O. H. Frankel (1951–62), much more emphasis was given to longer term, more fundamental research of relevance to agriculture. Strong groups were built up in such disciplines as genetics, microbiology, plant physiology and biochemistry, and the Division grew rapidly in size and facilities.

Plant breeding continued on tobacco and several pasture plants, as well as on crops such as safflower and lucerne. A single gene controlling the relative proportions of oleic and linoleic acid was found in safflower, the forerunner to many recent programmes on selection for oil quality. Attention was also given to background research in cytogenetics, induced mutations, and quantitative and biochemical genetics, including the first identification of a biochemical mutant among higher plants. Current emphasis is on the molecular organisation of the genome and particularly on the role of repeated sequences in DNA, whose pattern can be used, for example, to identify the individual rye chromosomes in *Triticale*.

Research on symbiotic nitrogen fixation by legumes has resulted in better understanding of many aspects of the process, such as the genetic and immunological variation within the bacteria, the structure of nodules and the functioning of nitrogenase, the role of the nodule pigment leghaemoglobin, the selection of more effective strains of *Rhizobium*, and the development of better methods of seed inoculation. Whether symbiotic nitrogen fixation can be extended to crops other than legumes is a question many research groups are considering, and our Divisional research has offered some hope in this context; an effectively nodulated non-legume has been found, and rhizobial nitrogen fixation in the absence of plant cells has been shown to be possible on a defined medium. Clearly, the rhizobia possess all the genetic information for the biosynthesis of nitrogenase, and their possible association with non-legumes can be contemplated.

Photosynthesis, the process on which all agricultural productivity depends, has also been a major topic of Divisional research in recent years. It has been explored at all levels of

After the establishment of the kinetic orders of the catalysed and uncatalysed reactions a mechanism consistent with all the results was deduced. The novel concept was that the rate controlling step was the reaction of a $[RCOO^- \ldots RCOOH_2^+]$ ion pair with ROH (ref. 3).

The clarification of the kinetics and mechanism of esterification and polyesterification reactions and the presence of microgel in alkyds was of considerable scientific value. In addition, the demonstration of microgel formation and the explanation of the lack of reactivity of some hydroxyl groups in alkyd resin was of great practical importance. Because alkyd resin solutions which contain microgel have the high molecular weight polymer present in dispersion these resins offer advantages in a paint over an alkyd in which the polymer is in true solution. Foremost among these are lower viscosity at the same solids content and faster drying in air drying formulations[2].

These advantages were such that the paint company which had presented the original problem set about deliberately to make alkyd resins with high microgel content and now have a patented method of producing a rapidly drying alkyd paint rich in microgel. In addition, the knowledge gained during this study, and subsequently published, made possible greater understanding of alkyd resin manufacture and consequent improvement in quality of the alkyd paints made by all manufacturers.

Another fundamental study of polymer chemistry which is finding important practical use was the investigation into novel isocyanates used initially for shrinkproofing, and recently for conferring 'permanent press' qualities on woollen garments. This investigation and its practical application was performed by the Divisions of Protein Chemistry and Textile Industry of CSIRO's Wool Research Laboratories.

The surface of a wool fibre is covered with scales oriented in one direction, and so in a textile the wool fibre can move more readily in one direction. When the textile is washed, the relative movement draws the fibres together and can also cause the development of slack sections. The two effects combined produce felting, shrinkage and distortion of garments. Relative movement between fibres can be prevented by interfibre bonding or by masking of the scales on the surface of the fibres. The component divisions of the Wool Research Laboratories have been working for many years on this problem and have developed a number of useful processes. The method most widely used to prevent shrinkage is to apply partly polymerised materials (prepolymers) either from solution in organic solvents, or preferably from aqueous systems followed by curing of the coated fibres or fabric, to complete the formation of an insoluble polymeric film.

Isocyanate prepolymers are used in CSIRO's current permanent press process[4] which has been in wide use in Australia since 1971, especially for service uniforms. But because of the reactivity of the isocyanate groups with water the prepolymers must be applied to finished garments from organic solvents in anhydrous conditions in a modified drycleaning machine and this has limited the commercial application of the process. In an attempt to develop systems which could be applied from water, methods were sought for masking the isocyanate groups, so that they are stable in water but still able to undergo subsequent polymerisation. Research starting from some early German work on the reaction of sodium bisulphite with simple model isocyanates in water demonstrated that these groups in isocyanate prepolymers can be masked by forming bisulphite adducts if alcohols are used as solvent[5]. The products are water soluble and stable to storage in aqueous solution. After the aqueous solution of the polymer is applied to wool the coating is cured as a result of the polymerisation of the isocyanate entity. This work has led to the Sirolan BAP[6] process which is extremely simple; the polymers are applied by padding using conventional textile equipment and then cured by drying. The process has been thoroughly proved in extensive industrial trials carried out in collaboration with the Australian Wool Corporation and the New Zealand Wool Board, and is expected to be used widely to impart machine washability to knitted fabrics, thus increasing the market acceptability of wool, one of Australia's most important export commodities.

Polymers for water treatment

In the group concerned with water purification research in what is now the Division of Chemical Technology, interest in the area of carbon and ion exchange led to the proposal of electrically regenerable ion-exchange systems based on semiconducting polymers. This followed a detailed study of the chemistry of carbon surfaces, in which it was shown that many carbons are organic polymers containing functional groups. The principle of electrical regeneration was shown to be sound, but space charge effects were observed which made it improbable that high exchange capacities

Table 1 Polymer research topics in CSIRO

Division	Topic
Forest Products (1927–71)	Structure of eucalypt wood; hardwood pulping processes; lignin; polysaccharides
Industrial Chemistry (1941–58)	Phenol/formaldehyde plastics; kinetics of the phenol/formaldehyde reaction; tannin–formaldehyde adhesives; reinforcing fillers for rubber; carbon black chemistry; conducting polymers
Physical Chemistry (1958–66) Applied Chemistry (1966–73) Chemical Technology (1974–)	Thermally regenerable ion-exchange resins, polymers for water treatment; magnetic filter aids.
Applied Mineralogy (1964–70) Applied Chemistry (1970–73) Applied Organic Chemistry (1974–)	Clay minerals and polymerisation; polyesterification; treated fillers for plastics; controlled release coatings for ruminant foods; cyclopolymerisation; synthetic antigens; chelating polymers
Protein Chemistry Textile Physics Textile Industry (1950–)	Structure of wool and wool proteins; polymeric coatings for wool; mechanical properties of fibres and fibre assemblies; polymeric coatings for leather
Food Research	Gas permeability of polymer films
Chemical Engineering	Rheology of polymer solutions and melts; polymers for boiler scale prevention
Building Research	Plastics in building; weathering of plastics and paints; fire resistance of plastics; mechanical properties of wood; tannin/formaldehyde adhesives

could be achieved when a large number of sites were present.

The realisation that heat would regenerate the resin more readily in certain systems has led to a new concept in ion exchange. Sirotherm (ICI Australia) resins were developed around the concept of regeneration with hot water rather than chemicals and their use has now reached the commercial stage with a plant built by ICI Australia Ltd to desalt 600 m^3 d^{-1} of low salinity water in Adelaide. A vigorous polymer synthesis programme has been maintained during the development stage, with the preparation of many resins of the weak electrolyte type. The acidic resins used are based on acrylic or methacrylic acid; a wide range of basic resins such as *N*-substituted polyvinylbenzylamines, polyvinylamines, polyaminoethylmethacrylates and polyallylamines have received attention. In addition, there has been the need to utilise microparticles to achieve sufficiently rapid rates of reaction. Ways of forming these into easily handled composite particles by various means, using polymeric multiphase systems, have been investigated.

When the commercial production of Sirotherm resins was first considered, it was realised that expensive initiator systems such as ultraviolet or γ radiation used during initial development needed to be avoided The Division of Applied Organic Chemistry developed chemical initiation systems for the possible basic components. During this study it was shown that in the polymerisation of diallylamines the ring size formed during the cyclopolymerisation was important in determining the properties of the resin. In an intensive study the polymers were examined by ^{13}C nuclear magnetic resonance to determine the presence and proportions of five-membered and six-membered ring structures and unchanged allyl residues The structures of the reacting radicals and polymers were determined by electron paramagnetic resonance both on polymerising systems and model compounds. This study has shown that polymerisation of substituted diallylamines gave linear saturated molecules containing predominantly five-membered ring structures[7] whereas earlier workers in the field suggested that six-membered rings were formed. It has also been shown that kinetic and steric factors played a greater part in determining polymer structure than did thermodynamic stability. The knowledge gained in this study has led to simple methods of control of ring size and basicity during the production of basic ion-exchange resins from substituted allylamines.

Microparticles were needed in the thermally regenerable ion-exchange process to ensure rapid rates of ion-exchange. The concept of magnetically flocculating microparticles to ease the difficulty of handling them has led to novel engineering procedures for standard ion-exchange processes. The magnetic polymers are prepared by embedding iron oxide in the resin particle. Both homogenous and shell materials have been prepared which can contain carboxylic acid, sulphonic acid, amino, or quaternary ammonium groups. Magnetic polymers devoid of functional groups have been tested successfully, in hydrophilic forms as magnetic filter aids. The high voidage of the flocculated magnetic polymers makes for an ideal filter bed, which has the advantage that it is easily recoverable and can be used over many cycles. Hydrophobic versions have been used in the collection of oil spills, with the oil being absorbed into the voids in amounts up to 20 times the volume of the magnetic polymer itself. Many applications are envisaged for these magnetic polymers, particularly but not exclusively in water treatment and recovery.

Polymers for biological systems

A developing area of polymer research in CSIRO is the use of polymers *in vivo* for increasing the productivity of animals and for use in human medicine. The examples described here—controlled release and synthetic antigens—are directed towards increased wool and meat production.

It was found in the Division of Animal Physiology (now the Division of Animal Production) that certain amino acids give enhanced wool growth when injected into the abomasum (fourth stomach) of a sheep. They have no effect, however, when administered orally, probably because they are microbiologically degraded in the sheep's rumen[8]. A basic polymer which was insoluble in the conditions normally experienced in the rumen (24 h, *p*H 7, 37 °C) but soluble within 1 h in the abomasum (*p*H 3) was designed and synthesised. This polymer can be used to coat amino acids or any other food or drug to give protection from degradation in the rumen of any ruminant animal. Polymer synthesis including a fundamental study of copolymerisation of monomers with basic functional groups, methods of coating amino acids with the polymer, and methods of administration of the coated material to sheep were all investigated and a workable system was devised and licensed to an international company[9]. Coated feeds should shortly be marketed and, because of a shift in markets during the course of the project, are expected to find a major application in feedlot production of beef as well as for supplementary feeding of sheep to enhance wool growth.

Increased wool and meat production by the cutting of losses due to infertility and pest attack is the result expected from the current work on synthetic antigens designed to generate antibodies which bind non-protein molecules. There are two immediate uses for this work; first in the radioimmunoassay of ecdysones (arthropod moulting hormones) in connection with work on pest control, and second for the immunisation of sheep against "clover infertility disease" which is a major economic problem in the Australian sheep industry.

Moulting is an essential process in the development of arthropods and interference with this process, either by causing premature moulting or by delaying moulting would cause death and thus could be used to control insect, tick or nematode populations at an early stage of development. Successful use of this control method, however, can only come after a knowledge of the natural concentrations of hormones present at the various stages of arthropod development is obtained. To do this requires the measurement of minute quantities of ecdysones, and radioimmunoassay is by far the most convenient technique.

Synthetic antigens had been used to generate antibodies for the radioimmunoassay of ecdysones previously, but the ecdysones had been linked to bovine serum albumin by the functional groups of the tetracyclic body of the molecule. As a consequence the ecdysone was partly masked by the protein carrier and the resultant antibodies were unselective and unreliable. By masking the functional groups of the tetracyclic ring and attaching the ecdysone through a side chain to the high molecular weight protein thyroglobulin, an antigen which produces antibodies in the rabbit specific to the tetracyclic structure of the ecdysone molecule was produced This antibody can be used for radioimmunoassay of various ecdysones with the same tetracyclic structure, but is sensitive to small changes in the tetracyclic part of the ecdysone, and consequently gives very reliable results.

The incidence of "clover infertility disease" in sheep is correlated with the presence in pasture plants of the phyto-oestrogen, formononetin, which is metabolised to equol in the rumen of the sheep[10]. It is now known that it is equol which is responsible for the oestrogenic effect of ingesting clover. For this reason a programme to try to immunise sheep against equol was initiated. In the work so far, derivatives of equol conjugated, at a position remote from the main functional groups, to bovine serum albumin have caused the formation of high levels of antibodies specific to equol in both rabbits and sheep. The results have been sufficient to justify an extensive field trial of immunisation with these synthetic antigens on sheep in West Australia where the problem is greatest.

A number of proteins have been used successfully as carriers in the synthetic antigen conjugates but they all suffer from problems of, for example, hydrolytic stability. Work is in progress to use synthetic polymers as carriers in these antigens. The control available over polymer composition and molecular size as well as greater stability should enable the current problems with protein conjugates to be overcome and should extend the use of synthetic antigens into other areas.

As illustrated in the examples discussed here, CSIRO polymer research is operating in many areas and at various scientific levels. The overall philosophy of this research which is similar to the approach used in other fields, is that the problem under investigation should be studied in sufficient depth to gain a proper understanding of the principles of the process. The value of this approach has been proved in many areas where not only the original problem was solved, but new lines of investigation and new applications for the knowledge gained have resulted.

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Australian journals of scientific research

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Since the Second World War the growth of scientific research in Australia has led to the creation of a group of nine national journals. CSIRO has had a major part in the creation, funding and running of these journals.

AUSTRALIAN scientists had few problems in finding suitable journals in which to place their research papers up to 1939. Output was small and it was easily absorbed by existing journals —mainly British and American and those few publications which had grown up in Australia during and after the First World War. The latter were primarily concerned with agricultural, medical and veterinary science. The best known are the *Medical Journal of Australia* (launched in 1914), the *Australian Journal of Experimental Biology and Medical Sciences* (1924), *Australian Veterinary Journal* (1925), the *Journal of the Australian Institute of Agricultural Science* (1934) and *Australian Forestry* (1936).

The Second World War was responsible for an enormous expansion in scientific activity in Australia. The Council for Scientific and Industrial Research (CSIR) with a total staff of 400 in 1937 had grown to 2,000 by 1946 It was evident to David Rivett (the wartime Chief Executive Officer of CSIR) and to some of his senior scientists that the traditional methods of disseminating research results were inappropriate to post-war conditions. Papers which fell outside the interests of existing journals tended to be published as CSIR Bulletins. Their content was, however, so diverse that distribution had become a problem to CSIR and their classification a headache to the libraries which received them. In 1947 CSIR, supported by the Australian National Research Council (ANRC), decided to create a national journal, the *Australian Journal of Scientific Research*. It was to be divided for convenience into two sections: Series A for the physical sciences and Series B for the biological sciences.

Dr N. S. Noble was appointed Editor by the two bodies and an Editorial Board comprising Professors W. J. Dakin, E. J. Hartung, L. H. Martin and J. G. Wood was set up. The first journals appeared in 1948 on a quarterly basis. In this first year 36 papers were published in Series A and 26 in Series B. The Editor acted as both Executive Officer and Chairman of the Editorial Board—a dual responsibility which was difficult to discharge, but which was later resolved when an independent Chairman was appointed by the sponsoring bodies.

The Editorial Board's functions were defined as follows: first, 'To take responsibility for acceptance and rejection of papers submitted to the *Australian Journal of Scientific Research*.' Second, 'To advise the Editor on matters of editorial policy.' Third, 'To advise CSIR and ANRC on matters affecting the publication of the *Australian Journal of Scientific Research*.'

All papers were refereed and contributions were accepted from any scientist regardless of institutional affiliation whether university, state or Australian instrumentality or industry. Originally about 60% of the submissions came from within CSIR—a proportion that has steadily decreased over the years to 25%.

By 1949 there was already some evidence of the problems which beset nationally sponsored journals. The *Australian Journal of Scientific Research* was tending to become an omnibus publication which even then had little appeal to the fast-fragmenting scientific community. Key[1] representing the views of CSIRO (CSIR changed its name to the Commonwealth Scientific and Industrial Research Organisation in 1949) scientists said that this led to "a general undervaluation of Australia's contribution to science". A part answer to this was the creation of three more journals: *Australian Journal of Agricultural Research*, *Australian Journal of Applied Science* and *Australian Journal of Marine and Freshwater Research*. These were sponsored entirely by CSIRO as the ANRC was not invited to collaborate in their establishment or control and the Editorial Board of the *Australian Journal of Scientific Research* was not concerned with them in any way.

An Editorial Advisory Committee, however, was appointed for each journal by the Executive of CSIRO. It was their responsibility to recommend acceptance or rejection of papers submitted for publication. Although printing difficulties and shortages of paper at the time made it impossible for the publisher to solicit contributions from outside CSIRO, papers were never rejected on those grounds alone.

Criticism of the nonspecific titles of three of the journals continued. How was it possible to decide whether a paper on plant–soil relationships was agricultural, applied or just scientific research?

By 1952 the Editorial Board decided that enough material of good quality was coming forward for Series A to be divided into two parts, namely the *Australian Journal of Chemistry* and the *Australian Journal of Physics*. The Executive of CSIRO decided to make the change in 1953 and invited the Royal Australian Chemical Institute to collaborate in the publication

Table 1 Summary of statistics for 1975

Australian Journal of (issues)	Pages published	Published papers	Rejected papers	Lapsed papers
Agricultural Research (6)	1,079	107	25	19
Biological Sciences (5)	573	60	22	7
Botany (6)	973	62	13	2
Chemistry (12)	2,744	331*	115	16
Marine and Freshwater Research (3)	428	36†	7	1
Physics (6)	1,043‡	80‡	38	7
Plant Physiology (4)	668	58§	15	6
Soil Research (2)	238	21¶	13	2
Zoology (4)	842‖	46‖	12	8
Total (48)	8,588	801	260	68

*Includes 87 short communications.
†Includes 11 short communications.
‡Includes 5 short communications and 5 supplements, 328 pages.
§Includes 6 short communications.
¶Includes 1 short communication.
‖Includes 2 supplements, 234 pages.

of the *Australian Journal of Chemistry* and the Institute of Physics (Australian Branch) in the *Australian Journal of Physics*. The Australian Institute of Agricultural Science and the Australian Veterinary Association had previously been invited to collaborate with the *Australian Journal of Agricultural Research*. As Series B could no longer continue without its partner, the journal was renamed the *Australian Journal of Biological Sciences*. In an attempt to answer all the previous criticisms the Executive of CSIRO established two further journals, the *Australian Journal of Botany* and the *Australian Journal of Zoology*, to provide outlets for the more descriptive papers in these disciplines.

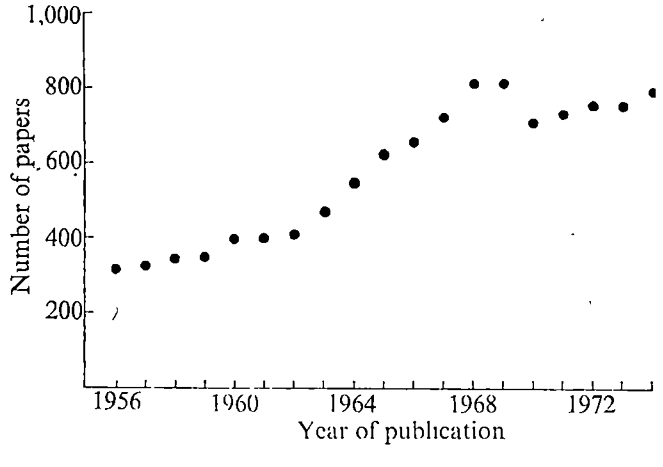
Extension of functions of the Board

The ANRC agreed to collaborate with CSIRO in sponsoring all the eight research journals which were to be published from 1953 onwards. The Editorial Board's function was amended to enable it to advise CSIRO and ANRC and assist the Editor in the publication of the eight journals. Editorial Advisory Committees were appointed for each journal and members of the Editorial Board were asked to serve on appropriate advisory committees.

The Australian Academy of Science

In 1957, the Australian Academy of Science agreed to fill the position left vacant by the disbandment of the ANRC in 1955 so that it now acts as a joint sponsor of the journals with CSIRO. A Board of Standards was set up to control editorial policy. The Board comprises a Chairman, a nominee of the Academy, a nominee of CSIRO, the Chairman of the Editorial Advisory Committees and the Editor-in-Chief. Its functions are spelled out in the Academy's Yearbook[2].

Fig. 1 Number of papers published annually since 1956.



Subsequent developments

In 1963 the *Australian Journal of Soil Research* was added to the series and one year later the *Australian Journal of Applied Science* ceased publication through lack of support. Dr Noble, who had seen all these developments and who had been responsible for the quality of the textual matter and high standards of presentation, retired in 1963 to be succeeded by Mr A. E. Scott who had been his senior editor. The journals grew steadily in stature under Mr Scott's stewardship until his retirement in 1971. Following several years of discussion with biological scientists, the Board of Standards recommended the creation of the *Australian Journal of Plant Physiology* in 1974.

Thus there are now nine journals in the series, whose current vigour can best be summarised by the publication statistics shown in Table 1 and the pattern of growth shown in Fig. 1. The number of papers published in 1975 was 500 more than in 1953 and 1,250 papers are now submitted annually. In the past 18 months there has been an abnormal increase in their number, partly because of difficulties being experienced in publishing outside Australia. Page charges of up to $100 are now being quoted by some US publishers and many journals are having to decline foreign papers.

Problems associated with national journals

It has always been difficult to persuade scientists in countries with small populations to support their local publishers. The problems have been discussed in some detail by Gregory[3] and Lim Huck Tee[4]. Australian scientists were no different, particularly in the first few years. Many considered that their work might be suspect if it were published in an Australian journal, while others rationalised their distrust by pointing to inadequate distribution of the journals and lack of awareness of their existence among scientists overseas. Even the titles of the journals have been criticised at times on the grounds that they represent an insular attitude to research, although it is difficult to see how one can differentiate between, say, one journal of botany and another without some adjectival label. Somehow names like *Helvetica Chimica Acta* and *J. Am. Chem. Soc.* have not prevented journals associated with particular nations or societies from being considered international in every sense.

The commonest complaint still heard is that specialists must publish only in specialist journals. The difficulty is that national journals tend to cover a broad spectrum of topics unless there is an active group of scientists of world renown working in the country in question who are able to attract contributions from overseas. The *Australian Journal of Plant Physiology* seems to have achieved this happy state in less than two years although it must be remembered that the *Australian Journal of Biological*

Sciences published first-class papers in plant physiology for many years before the new specialist journal appeared. Specialisation in a national journal can also have its drawbacks. The *Australian Journal of Physics* has had a reputation (rather unfairly one feels) for specialising in radiophysics and high energy physics. This has persuaded some physicists that the editors are not interested in publishing in such areas as solid-state physics or optical astronomy. These prejudices can be broken down only slowly with the help of the advisory committees and a welcoming editorial attitude. On the other hand, acceptance of substandard papers in 'new' disciplines will certainly not achieve the required result.

The efficiency of secondary publication services these days puts any journal in the public eye more effectively than ever before An author's search for visibility through large circulations is now no longer necessary. The general decline in the world's postal services has ensured that a scientific contribution from Australia is noted in current awareness documents like *Current Contents* long before the journal itself reaches the ultimate reader. Requests for reprints often arrive before the reprints have left the publisher. It is, of course, the publisher's responsibility to ensure that his journals are sent promptly to the leading abstracting organisations and that they are laid out in the most appropriate way for easy incorporation into the appropriate data bases and secondary journals The Australian Journals of Scientific Research were redesigned in 1974 with this in mind. Each contribution is now headed by full bibliographical details and an indicative abstract, while information that is irrelevant for retrieval purposes has been relegated to footnotes or to the end of articles

Recent studies of citations indicate that the Australian journals rank well in their various categories. Thus the *Australian Journal of Biological Sciences* ranked thirtieth in Garfield's analysis of most frequently cited botany journals[5] (this was before the *Australian Journal of Plant Physiology* appeared).

The *Australian Journal of Physics* is creditably placed among the leading astronomy journals even though it carries only a small proportion of radiophysics papers these days. In agriculture, the *Australian Journal of Agricultural Research* is listed thirty-eighth in the top 100 journals included in a recent Science Citation Index analysis[6].

Advisory committees

Each journal in the series has a separate editorial advisory committee. The chairman of each committee is appointed jointly by the Academy of Science and the Executive of CSIRO and automatically becomes a member of the Board of Standards.

Committee members are chosen by the Board of Standards with an eye to ensuring as wide a coverage as possible of the topics likely to be found in the journal. It is their responsibility to keep a watching brief on the standards of the papers and to act as arbiters when referees fail to agree on the worth of any particular submission They are also available to help the editors choose referees. Although the committee of each journal normally meets only once a year, it is continually at work encouraging people to submit papers to the journal, watching for new areas of research that would be of interest to the journal and doing those many vital and unpaid tasks that are the lot of any publishing committee. There are currently sixty-two scientists employed in such activities for the nine journals.

Table 2 Overseas recipients of Australian journals

USA	2,560	France	228
UK and Eire	1,052	South Africa	196
Canada	514	Italy	170
Japan	438	Netherlands	154
India	425	Pakistan	151
USSR	355	Indonesia	145
Germany (all)	349	Malaysia	116
New Zealand	230		

Referees

The standard of any scientific journal ultimately rests on the quality of its refereeing system. All papers submitted to the journals are refereed thoroughly by independent authorities selected by the editors and their advisory committees. Naturally, most referees are chosen from research centres in Australia and New Zealand, but there has been, over the past four years, a much greater dependence on referees in Europe and North America At the same time, the total number of referees used has expanded dramatically. The use of such overseas expertise has had a marked effect on the quality of the papers submitted from outside the immediate catchment area. The newest of the journals, the *Australian Journal of Plant Physiology*, has already attracted the attention of authors from Japan, Germany, the United States and Israel.

The publishing office

Production of the set of national journals in East Melbourne is unique. Even the Canadian journals are not compiled in such a closely integrated manner and the Australian journals have the added advantage of being printed by the publisher. The editors —each specialists in their own right—are in daily contact with each other. If a mathematical problem arises in a biological paper there is immediate help at hand from say one of the physics editors or if a biochemical paper contains an unusual compound its structure or name can be checked by a specialist in chemical nomenclature

Production methods are continually being improved by the use of modern printing techniques, the inclusion of computer-generated tabular matter and better methods of binding and packaging The only backward area—and it seems to be the one that defeats all publishers today—is cheap and swift distribution of the final product to the readers. But every international publisher believes that his own postal system is the worst in the world.

Relations with the scientific community

The close relations with the Royal Australian Chemical Institute, the Australian Institute of Physics, the Australian Institute

Table 3 Distribution of publishing time from receipt of manuscript

Australian Journal of	Elapsed time (months) 3	4	5	6	7	8	9	10	11	12	> 12	Total number of papers
Chemistry	5	50	115	100	27	19	10	1	3	2	1	333
Biological Sciences	5	8	14	40	28	14	6	9	2		4	130
Agricultural Research		4	14	30	17	12	2	4	4	2	5	94
Physics		2	11	17	18	13	10	8	1	1	3	84
Zoology	8	6	4	7	3	1	1					30
Botany	1	2	5	2	3	7	2	1			1	24
Marine and Freshwater Research	3		3		1		1	1	1		1	11
Soil Research		1	1	1	1	2	2		3			11
Total	22	73	167	197	98	68	34	24	14	5	15	717

of Agricultural Science, the Australian Veterinary Association and the Australian Society of Soil Science have been mentioned already. More strenuous efforts have been made recently to encourage members of other scientific societies to consider the journals as natural homes for their own research papers. These overtures are being gratefully received at a time when most societies are finding it increasingly difficult to publish more than a simple news bulletin because of the soaring costs of printing and administration. Moreover, many journals published in other countries are either resorting to high page charges or otherwise discouraging Australian authors by restricting the length of papers or increasing the period that a paper remains 'in press'.

Concessional subscription rates are now offered to the members of sixteen scientific societies in Australia at prices which represent little more than the run-on cost of the journals plus an allowance for postage and handling. The scheme has been well received by the societies and their members.

It has been mentioned already that support for the journals has been canvassed among all scientists in Australia and not merely among CSIRO staff. Now half the published papers come from Australian universities, one-quarter from CSIRO and the rest from other institutions in Australia and abroad.

In 1975 some 48 issues of the various journals together with seven supplements were published. Over 800 papers were published in 8,588 pages and 14,100 sets of the various journals were distributed in more than 100 countries. The main recipients of the journals outside Australia are listed in Table 2.

The most recent analysis of publication times is shown in Table 3. The median time from receipt of manuscript to publication is six months based on a total sample of 717 papers taken from eight of the journals. The *Australian Journal of Chemistry* has the best record (5 months) because it is the only publication in the series that is published monthly. All the others are published less frequently (Table 1). It takes about three months from acceptance for a paper to appear in most of the journals. This figure is fairly constant as a result of the tight schedules kept by CSIRO's printers. Refereeing is responsible for the greatest part of the variation in publication times, particularly when manuscripts have to travel long distances. It is worth noting, however, that referees in Europe and the USA generally do everything they can to speed up the refereeing process by returning their reports promptly.

The future

The scientific journal in its present form will not disappear overnight. Too many conservative forces are at work for this to happen. Agreement between authors, publishers, printers, librarians and readers is necessary before a more efficient system of publication, storage and dissemination of primary research results is likely to be accepted. But our problems are no different from those of others engaged in generating scientific knowledge. How to do more with shrinking resources in real terms? Luckily, the tremendous increase in output during the past few years seems to be slowing down, but this alone will not save the publisher from the effects of inflation on everything he does. More use must be made of storage centres for extraneous data not needed by the majority of readers; substandard submissions must be ruthlessly rejected or sent back to authors for restyling; cheaper and faster methods of production must be sought, second thoughts by either editors or authors cannot be tolerated at the proof stage; indeed, perhaps the time has come when proofs should not be sent to authors except in special circumstances, and parts of the journal may be issued in microform only. CSIRO's computer with its microfilm output equipment (a COMp 80 phototypesetter) is now being used to produce camera-ready copy for the supplements to the *Australian Journal of Physics*. The same data can be published in microfiche with little more than a change of camera. Experiments have shown that it is feasible to set complex mathematical text more economically by computer than by anything other than strike-on systems such as the IBM composer. Our aim, however, remains to produce text of high quality with first class typography in the most appropriate format.

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articles

On the mechanisms of lunar regolith glass particle formation

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The discovery of many particles of glass in the lunar regolith has resulted in various hypotheses for their origin[1]*. Based on previously unpublished work*[2]*, we here give an analysis of the principal mechanisms of their formation and evolution.*

FIRST, we show that the condensation of vapour created by the impact of a meteorite does not make a noticeable contribution to particle formation but spraying does. For this we consider the size distribution of lunar glasses[3–5], which has a bimodal character, with a sharp left maximum in the range of 0.1–10 µm and a weak right maximum in the range of 100–500 µm (see Fig. 1) and compare it with the distribution function of meteor material. The linear dimensions (r) of condensate particles are proportional to those of the initial meteorite (R) (ref. 7): $R=Kr$ and $K\simeq 10^3$. This gives a characteristic size of condensed particles of $\sim (1\text{–}5)\times 10^{-3}$ µm, that is, almost atomic size. The

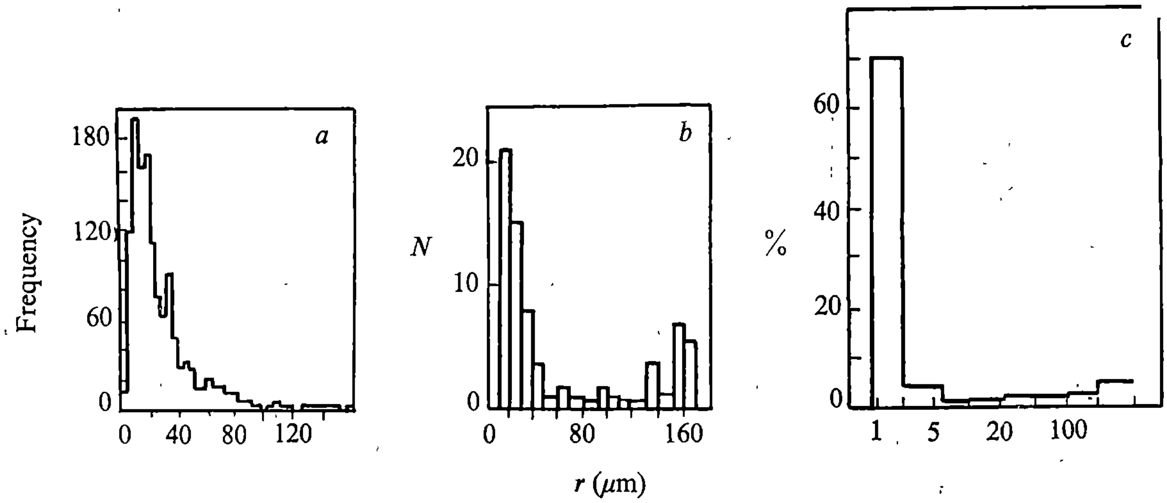

Fig. 1 The size distribution of lunar glasses. Data in *a* are taken from ref. 5, in *b* from ref. 4 and in *c* from ref. 3.

spraying of the impact-melted material gives an analogous coefficient $K'\simeq 10$, leading to characteristic sizes $r\simeq 0.1$–1 μm (ref. 2). As far as we know the finest-grained glass particles have been measured in detail only in ref. 6, and the data agree with this result.

The characteristic particle size (d) can be estimated in another way from the cohesive characteristics of lunar regolith[2]

$$d\simeq 2r_m(\sigma_t/\sigma_m)^{\frac{1}{2}}$$

where r_m is the intermolecular interaction distance, ~ 10^{-7} cm, σ_t is the individual particle strength, ~ 10^9 dyne cm^{-2}, and σ_m is the macroscopic shear strength of lunar regolith. Taking $\sigma_m = 5\times 10^3 - 5\times 10^4$ dyne cm^{-2} gives $d\simeq(0.5\text{–}2)\times 10^{-1}$ μm (ref. 8). This value partially overlaps the previous estimate and therefore corroborates the idea that the spraying mechansim during meteorite impact is predominant in the formation of small glass particles.

The large particles of the right mode, ~ 100–500 μm, can form only as a result of slow endogenic processes and thus should be 'older' than the small ones.

The spraying mechanism

Regular particles are formed from the decay of irregular jets of melted material by the interplay of inertia and surface tension. Balancing these forces brings about some characteristic maximum stability size r_{st} that depends on characteristic process rates. The left and right peaks in particle size distribution are related to the high velocity (meteorite) and low velocity (endogenic) processes, respectively. Indeed, if $(\overline{v^2})^{\frac{1}{2}}$ is the r.m.s. internal velocity of a liquid particle and α is the coefficient of surface tension, then we get

$$\rho\frac{\overline{v^2}}{2}\simeq\frac{\alpha}{r_{st}}$$

(ρ is the density of the liquid). It would be natural to assume that $(\overline{v^2})^{\frac{1}{2}}$ is related to the velocity, u, of a typical particle, size l, so that the ratio $(\overline{v^2})^{\frac{1}{2}}\sim ur/l$. Thus it follows that

$$r_{st}\simeq\frac{l}{u}\left(\frac{2\alpha}{\rho}\right)^{3/2}$$

One can easily calculate that $r_{st} < 10$ μm for the velocities and dimensions characteristic of meteorite impacts and all the particles generated in such a way are in the region of the left mode.

The shapes of the glass beads

The relationship between the elongated (ellipsoidal dumb-bell) and spherical shapes of particles will be considered next. The drops will vibrate (if energy of their internal movements is not sufficient for decay) as

$$X = X_0\exp(-\gamma t)\sin(\omega t+\delta)$$

where X is the typical elongation of a drop, $\gamma = \nu/\rho d^2$ is the attenuation constant of vibration (ν is the viscosity)[9], and

$$\omega \simeq 2\pi(\sigma/\rho V)^{\frac{1}{2}}$$

$V = d^3/2$ is the drop volume in the equilibrium position.

The average degree of elongation e at some time t can be determined from X^2 by averaging over the random phase, δ

$$e^2 = \langle X^2\rangle_\delta = \frac{X_0^{\,2}}{2}\exp(-2\gamma t)$$

Because of the exponential dependence of viscosity on temperature, it is assumed that at a certain moment $t = t_s$ the particle solidifies and 'memorises' its shape.

It can be shown easily that $t_s \simeq d$ and thus $\gamma \simeq d^{-2}$; then

$$e = \frac{X_0}{\sqrt{2}}\exp\left(-\frac{\text{const.}}{d}\right)$$

Thus, the average elongation of particles, e, markedly decreases with decreasing particle size; just this has been observed in lunar particles[3].

The popular theory that rotating drops should form elongated and dumb-bell[10–12] shaped particles is not valid. Rotating particles will sometimes acquire an S shape.

Let τ be the time during which a spherical particle is elongated to the length $2l$ with mean velocity $v = l/\tau$. If ω is the angular velocity of particle rotation then $a_\perp = 2\omega v$ is the Coriolis acceleration, and $S = a_\perp\tau^2/2$ is the transverse deformation. On the other hand, the longitudinal acceleration is $a_\parallel\simeq\omega^2 l$ and $l\simeq a_\parallel\tau^2/2$, hence one gets the value of $S/l = \omega\tau\simeq\sqrt{2}$. Since $S/l > 1$, bending of the rotating particle is quite significant. Rotation about a dumb-bell symmetry axis also does not bring about the dumb-bell shape because of the instability of forms that are thus generated[2].

Thus, the variation principle for determining static equilibrium shapes[10,11] cannot be used. A great number of elongated particles could be generated as a result of vibrations, but only a few of the crescent particles observed could be formed by rotation.

Pools of melted magma

We can also show that no 'pools' or 'lakes' of melted magma are formed by meteorite impact on the crater floor, which could have been the source of large particle formation. The velocity u of the medium dragged behind the shock wave is related to the specific energy by $\varepsilon \simeq u^2/2$; therefore $u \gtrsim (2\varepsilon_m)^{1/2}$ where ε_m is the specific melting heat $\sim 5\times10^9$ erg g^{-1} (ref. 7) with $u \gtrsim 10^5$ cm s^{-1}. It is clear that most material will be ejected a long way except for some from the region near the crater wall. The formation of a boundary layer can be easily shown to occur by making an estimate of Reynolds number for the given outflow ($Re > 10^3$) for craters with $D_s > 1$ cm. If we estimate the boundary layer thickness, δ_0, in a usual way, then $\delta_0 \sim 10^{-2}\sqrt{D_s}$ will be obtained where D_s (cm) is the crater diameter. Values $\delta_s = 1$ cm and 0.03 cm are obtained for craters with $D_s = 10^2$ m and 0.1 m respectively.

It is clear that the cooling layer $\delta_0 \sim 0.03$ cm thick will not flow down over the rough soil, and the layer $\delta_0 = 1$ cm for a 100-m crater will give the maximum depth for a pool $\sim$ 30 cm. These figures are gross overestimates, since the edge of the boundary layer has a higher velocity than the bulk of the material, which will join the disintegrated soil.

Thus, only vitrified surfaces are formed in meteorite impact, not 'pools' and 'lakes'. Therefore, the large particles of the right peak in the size distribution also cannot be formed. They must have been formed in endogenic volcanic processes.

Secondary particles

These very simple estimates show that new regularly shaped particles are not formed during the collision of secondary particles (meteorite interaction products) with the lunar surface. Indeed, such particles have a velocity, v, lower than the escape velocity ($v_e \simeq 2.4$ km s^{-1}). The corresponding energy, ε, in the shock wave is $\varepsilon \simeq v^2/2 \simeq 5\times10^9$ erg g^{-1} which lies on the boundary of specific melting heat. As is well known[7], an approximately tenfold energy storage is required for starting the spraying process, and melting is never experimentally observed for such velocities.

A simple model

We may construct an elementary model of the evolution of lunar surface material where a depth h is uniformly affected by systematic meteorite bombardment. The mass fraction of primary material is designated by μ_1, crushed material by μ_2 overmelted material by μ_3 and crushed glass by μ_4. Then they follow the following set of equations

$$\frac{d\mu_1}{d\tau} = -(\alpha+\zeta)\mu_1; \quad \frac{d\mu_2}{d\tau} = \zeta\mu_1 - \alpha\mu_2$$

$$\frac{d\mu_3}{d\tau} = \beta\mu_4 - \alpha\mu_3; \quad \frac{d\mu_4}{d\tau} = \alpha(\mu_1+\mu_2+\mu_3) - \beta\mu_4$$

where α, β, ζ are the relative volumes of 'pit', 'halo' and 'crushing zone', respectively. From ref. 2, we obtain $\alpha \simeq 10^{-2}$; $\zeta = 1-\alpha$; $\beta \simeq \zeta \simeq 1$, and τ is dimensionless geological time.

Assuming, for simplification, that $\beta = \zeta = 1-\alpha$ and choosing the initial conditions $\mu_2(0) = \mu_3(0) = \mu_4(0) = 0$ and $\mu_1(0) = 1$ the solutions of the equations are

$$\mu_1(\tau) = e^{-\tau}; \ \mu_2(\tau) = e^{-\alpha\tau}(1-e^{-\beta\tau})$$

$$\mu_3(\tau) = \beta - e^{-\alpha\tau} + \alpha e^{-\tau}; \ \mu_4(\tau) = \alpha(1-e^{-\tau})$$

From these solutions it follows that the events develop with two time constants $\alpha^{-1} \gg 1$ and $\beta^{-1} \simeq 1$. Primary material (μ_1) is rapidly (within time $\simeq 1$) changed to crushed primary material (μ_2), which grows up to $\tau \sim 1$, then slowly ($\sim\alpha^{-1}$) decreases because of the remelting of glass of regular shape (μ_3). These particles produce the crushed glass on bombardment, and its fraction μ_1 rapidly grows. With $\tau \gg \alpha^{-1}$ all the material is transformed into glass of regular (μ_3) and irregular (μ_4) shape, and $\mu_3 \rightarrow \alpha$, $\mu_4 \rightarrow 1-\alpha$.

Ratios of $\mu_1 : \mu_2 : \mu_3 : \mu_4$ are additional experimental chronological characteristics for lunar surface sites.

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Volatilisation of elements from melts of lunar material

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We discuss here the theory of the volatilisation of components of molten rocks and develop a model for this process, which enables us to draw some conclusions about such phenomena on the Moon.

CHEMICAL analyses of the surface of the Moon have shown that it is depleted in such volatile elements as Na and K when compared with similar terrestrial rocks. To explain this, two contradictory hypotheses[1] have been put forward: first, that the magmatic material is depleted in these elements because of volatilisation from magmatic melts on the surface of the Moon and second, that magmatic melts effused on the surface were primordially depleted in such elements. To elucidate this problem, numerous attempts have been undertaken to simulate the depletion process by heating melts of terrestrial rocks to high temperatures, both in a vacuum and in an atmosphere of inert gases[2-7]. There has been no adequate physical model, however, and it has been impossible to extend the experimental results to lunar conditions.

Here we develop a theory of volatilisation and show that the presence of oxygen has a decisive influence on the process kinetics, especially on the final state of the melts. This provides the basis for the laboratory simulation of volatilisation and also for an understanding of lunar processes.

Basic assumptions

Thermodynamically, a high-temperature melt placed in a vacuum chamber with 'cool' walls is a significantly non-equilibrium 'open' system. In such a system, the evaporation of a volatile component should proceed until it is fully evaporated. Yet, experiments have revealed the existence of plateaux in component concentrations which are stable even after long exposure. Moreover, the heights of the plateaux were found[6] to depend on the pressure in the experimental vacuum chamber, but since in the pressure range involved ($p < 10^{-3}$ mmHg) the return coefficient Z is $\ll 1$, these plateaux cannot be caused by the pressure-dependent return of volatile molecules to the melt.

The only possibility for explaining the data is that molecules of the volatile elements reaching the surface may either leave the melt or enter a non-volatile state through a chemical reaction. Oxidation is just such a reaction, the oxygen being adsorbed on to the melt surface from residual gas in the unit.

The model

For the mathematical investigation of the volatilisation process, the melt can be assumed, with reasonable accuracy, to be a regular solution[8] of dissolved Na (hereafter by Na we mean any volatile element). Denoting the volume concentrations (cm^{-3}) of Na in the melt by x_1 in the volatile and x_2 in the non-volatile states, one can deduce the following kinetic equations for the concentrations

$$\frac{dx_1}{dt} = k_{21}x_2 - k_{12}x_1 - j\exp(-\beta x_1)x_1 \tag{1}$$

$$\frac{dx_2}{dt} = k_{12}x_1 - k_{21}x_2 \tag{2}$$

where k_{12} and k_{21} are the rate constants for transition from state 1 to 2 and from 2 to 1, respectively. The quantity j characterising the rate of evaporation is

$$j = \frac{(1-Z)\alpha_V\mu}{h\rho(2\pi mkT)^{\frac{1}{2}}}\exp(A/RT)p_1(T) \tag{3}$$

In this case $p_1(T)$ is the vapour pressure for pure Na at temperature T, R the universal gas constant, μ and ρ are the molecular weight and density of the solvent, A the heat of solution of Na in the melt per unit of concentration, k the Boltzmann constant, m the mass of the Na atom, Z and α_V are the return and evaporation coefficients and h is the melt depth.

The value h is taken to be sufficiently small for the time of transport of the Na molecule to the surface, compared with the other characteristic times of the process, to be neglected. The exponent factor in equation (1) is

$$\beta = \frac{2A\mu}{\rho RT} \tag{4}$$

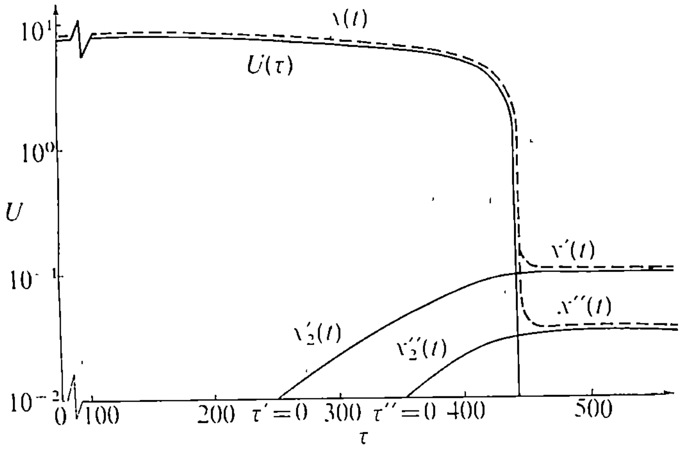


Fig. 1 $U(\tau)$, universal dependence, and $x'(t) = x_1(t)+x_2(t)$ and $x''(t) = x_1(t)+x_2''(t)$ dependences.

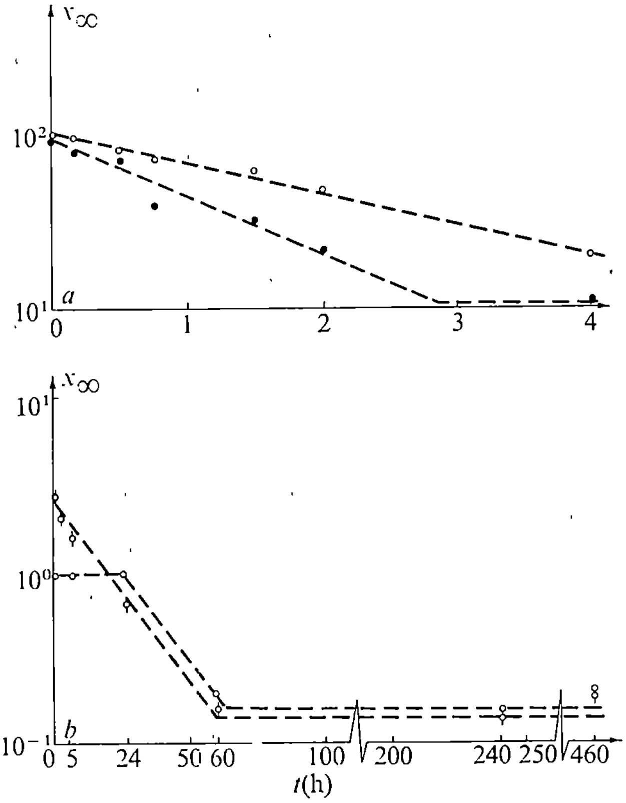


Fig. 2 Volatility kinetics for Na and K from the melt of the terrestrial rock in a vacuum, a, $T \simeq$ 1,050 °C; $p \simeq 10^{-6}$ mmHg (ref. 7) b, $T \simeq$ 1,150–1,175 °C; $p \simeq 10^{-4}$–10^{-5} mmHg (ref. 2); open circles with and without dot, K; closed circles and open circles with ticks, Na.

Solutions

An analysis of the solutions of equations (1) and (2) for $t \to \infty$ and of the experimental data shows that the inversion from the non-volatile state 2 to the volatile state 1 is practically absent for

$$k_{21} \ll j + k_{12}; \quad k_{21} \ll j; \quad k_{21} \ll k_{12}$$

After neglecting the terms proportional to k_{21} in equations (1) and (2) the system is solved in quadratures

$$\int_{x_{10}}^{x_1(t)} \frac{dx}{x[\exp(-\beta x)+(k_{12}/j)]} = -jt \tag{5}$$

$$x_2(t) = x_{20} + k_{12}\int_0^t x_1(t')dt' \tag{6}$$

In equations (5) and (6) we have labelled the quantities corresponding to the initial state by 0. For the qualitative analysis of the solutions (5) and (6), we neglect the term k_{12}/j in (5) and find

$$E_1{}^*(\beta x_1) = E_1{}^*(\beta x_{10}) - jt \tag{7}$$

where $E_1{}^*(z)$ is the integral exponent determined and tabulated in ref. 9.

Analysis

Formula (7) gives the universal curve of the dimensionless concentration as a function of dimensionless time, $\tau = jt$.

To the various initial concentrations $U_0 = \beta x_{10}$ correspond shifts of the reference of time to the universal curve (7) by the value $E_i^*(U_0)$. Figure 1 shows that the total concentration $x(t) = x_1(t)+x_2(t)$ has two types of kinetic dependence: a two-step and a one-step curve. The first is realised if $U_0 \equiv 2A\mu x_{10}/\rho RT \gtrsim 5$ while the second is realised when $U_0<5$. In particular, if $U_0<1$ then $x(t)$ tends to drop immediately after the period of exponential decrease of $x_1(t)$. These types of behaviour have been observed experimentally[2,3,6,7] (Fig. 2).

For investigating the pressure and time dependences we consider the case $U_0<1$ which was involved in these experiments. In this case $\exp(\beta x) \simeq 1$ and the equations (5) and (6) yield for $t \rightarrow \infty$

$$x_\infty = x_{20}+x_{10}[k_{12}/(j+k_{12})]; \quad x_\infty \equiv [x_1(t)+x_2(t)]_{t\rightarrow\infty} \tag{8}$$

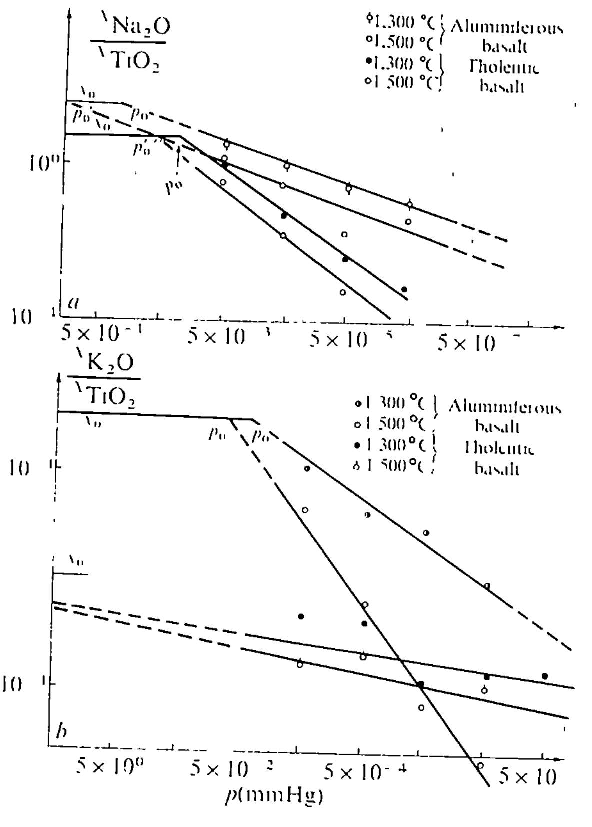
The two terms of equation (8) with $x_{20} \neq 0$ cannot give the observed power-type pressure dependence x_∞ (ref. 6 and Fig. 3). Therefore it is natural to put $x_{20} = 0$ in equation (8).

It can be shown that in the high vacuum region the pressure dependence of j is unimportant, therefore the dependence of x_∞ on p is determined by the value $k_{12}(p)$. Here the experiment gives $k_{12} \propto p^\gamma$, as in the absorption isotherm of Freundlich[10] for the concentration $n(p)$ of the molecules (atoms) adsorbed on the surface from the residual atmosphere.

It thus appears that the only possible explanation of the presence of the power-type pressure dependence is the assumption that Na is oxidised on the melt surface by oxygen sorbed on the surface. In this case

$$k_{12} = ap^\gamma \exp\left(-\frac{Q-q_v}{RT}\right) \propto n(p)\exp\left(-\frac{Q}{RT}\right) \tag{9}$$

Fig. 3 *a*, *b*, Volatility of Na and K from the melt of aluminiferous and tholeiitic basalts in vacuum (T = 1,300 and 1,500 °C) (ref. 6).



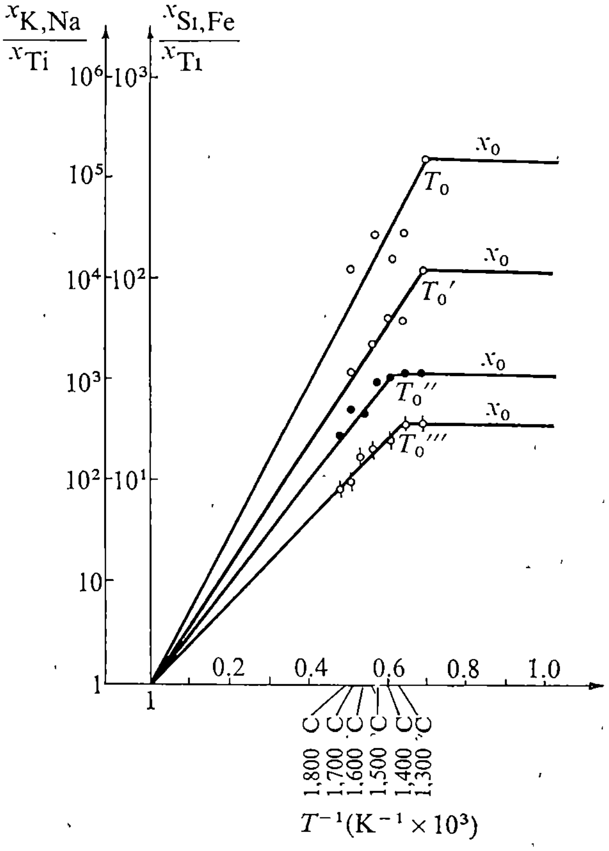


Fig. 4 Summary plot of the volatility dependence of Na, K, Si and Fe in vacuum ($p \leqslant 10^{-6}$ mmHg) from the melt of basalt[5,6]. A heat of reaction, ψ, is determined by the bend angle at the $x_\infty(T)$ curve $\psi_K = 31.2$ kcalorie mol^{-1}; $\psi_{Na} = 21.6$ kcalorie mol^{-1}; $\psi_{Si} = 5.14$ kcalorie mol^{-1}; $\psi_{Fe} = 5.0$ kcalorie mol^{-1}. ○, K/Ti: ⊙, Na/Ti; ●, Si/Ti; ⊙, Fe/Ti.

where a is a constant, q_v is the heat of adsorption (several units of kcalorie mol^{-1}) and Q is the energy of oxidation activation (of the same order as q_v (ref. 10)).

Let us factorise the main temperature dependence in equation (3)

$$j = b\exp\left(-\frac{L-A}{RT}\right); \quad b = b(T) \simeq \text{const.} \tag{10}$$

where L is the heat of evaporation of pure Na ($\simeq$ 25 kcalorie mol^{-1}). Now we can write for x_∞ the final expression

$$x_\infty = x_{10}\frac{k_{12}}{j+k_{12}}$$

$$= x_{10}\frac{ap^\gamma\exp(-(Q-q_v)/RT)}{b\exp(-(L-A)/RT)+ap^\gamma\exp(-(Q-q_v)/RT)} \tag{11}$$

From equation (11) it follows that there exist two pressure and temperature regions ($j \gg k_{12}$ and $j \ll k_{12}$) which account for the presence of specific kinks p_0 and T_0 on the curves $x_\infty(p)$ and $x_\infty(T)$ (Figs 3 and 4). Because $(L-A)$ is always > $(Q-q_v)$ in the low temperature region $T < T_0$, $k_{12} \gg j$ and $x_\infty \simeq x_{10}$, and practically no volatilisation occurs. In the region $T > T_0$, $j \gg k_{12}$ and volatilisation occurs rapidly, in which case

$$x_\infty = x_{10}(k_{12}/j) = x_{10}(a/b)p^\gamma\exp(-(Q-q_v+A-L)/RT)$$

which demonstrates the power-type pressure and exponential-type temperature dependence.

At reasonably high pressures $p \gg p_0$, $j \ll k_{12}$, volatilisation is almost complete and the pressure dependence disappears[6].

Lunar applications

Thus, the picture presented here facilitates explanation of all the peculiarities of the experimentally observed dependences of x_∞ on t, p and T in the region of low pressures and moderate temperatures. As regards volatilisation on the Moon, our theory is applicable when we may neglect transport processes, that is, in a fairly thin layer. In this case the estimate of U_0 using the parameters of the lunar material[3] indicates that only for $x_0 \leqslant 5\%$ could the exponential volatilisation regime $x_1(t) \simeq x_{10} \exp(-jt)$ have occurred.

Heating lunar material in a laboratory vacuum demonstrates that it is in the exponential kinetic volatilisation regime, and therefore that the process occurred on the Moon at $T > T_0$, which could have led to the complete disappearance of Na from the surface material if rather rapid cooling had not taken place. The observed low concentrations of free Na in lunar material[3] also point to the almost complete absence of oxygen in the ancient Moon's atmosphere. The emergence of the curves $x(t)$ on to the plateau obtained in a number of laboratory experiments[2,3,6,7] is a purely instrumental effect associated with the presence of oxygen in the residual atmosphere of experimental equipment. We note that the process considered here gives a possible mechanism for the 'disappearance' of oxygen and other active gases from the atmosphere of the Moon and planets: sorption on the melted material on the surface, with subsequent chemical bonding. For determining the partial pressure of oxygen in the Moon's atmosphere at the instant of cooling of the lunar material down to $T \lesssim T_0$, it is necessary to perform an investigation of volatilisation from lunar rocks in a vacuum-pumping assembly with $p \simeq 10^{-12}$–10^{-13} mmHg. If a plateau is revealed on the curve $x(t)$, then its height will define p_{O_2} in the Moon's atmosphere according to our theory. The suggested theory has been formulated for isothermal volatilisation and applies therefore primarily to exogenic (volcanic) processes accompanied by the formation of large accumulations of melted material. The common viewpoint, attributing volatilisation to meteorite collisions, is wrong, since they do not produce significant volatilisation of Na, at least in single collisions (see ref. 11). This results from the fact that in collisions relatively small[11,12] accumulations of liquids are formed, which solidify before significant volatilisation occurs.

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Partial sequences of 16S rRNA and the phylogeny of blue-green algae and chloroplasts

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Partial sequence analyses of 16S ribosomal RNAs of blue-green algae and chloroplasts reveal that blue-green algae are typically prokaryotic and related to the bacilli, that red algal chloroplasts are probably of blue-green algal origin, and that euglenoid and red algal chloroplasts may have arisen independently.

WE have begun to assemble sequence information on the 16S rRNAs of the blue-green algae and on the evolutionary homologues of these molecules in eukaryotic algae· chloroplast 16S and cytoplasmic 18S rRNAs. The assembled data should serve to determine the relatedness of blue-greens to bacteria, to establish taxonomic structure within the blue-greens, and to determine phylogenetic relationships between blue-green algae and chloroplasts

Blue-green algae are a major group of photosynthetic prokaryotes readily distinguishable from photosynthetic bacteria by their possession of oxygen-evolving photosynthetic mechanisms otherwise found only in eukaryotic algae and higher plants. There is little quantitative information on which to base measurements of the extent to which this group is related to any of the major groups of bacteria, either photosynthetic or non-photosynthetic. Woese and his collaborators[1–3] have shown the use of 16S rRNA sequence information (in the form of 'T_1 oligonucleotide catalogues') in establishing phylogenetic relationships among bacteria. Acquisition of similar information for blue-greens should allow their unambiguous positioning among the prokaryotes.

Classical taxonomies of blue-green algae rest on morphological characters perhaps suitable for the structurally differentiated multicellular filamentous forms but wholly inadequate for classification of unicellular species. The latter are likely to be of greater antiquity[5] and phylogenetic diversity, showing a range of DNA GC contents (35–71 mol %) nearly as large as that characterising the most distantly related bacteria (25–75 mol %)[4]. Quantitative treatments such as those now being developed by Stanier[4] and that which is possible for RNA sequence data are required for the construction of a rational taxonomy for this group

Recognition of the near identity of photosynthetic mechanisms in blue-greens and eukaryotic photosynthesisers underlies the now generally accepted notion that chloroplasts, the semi-autonomous, self-replicating organelles of eukaryotic photosynthesis, evolved from oxygen-producing photosynthetic prokaryotes resembling blue-green algae[6–10]. Two specific hypotheses concerning this evolution can currently be entertained· (1) the "endosymbiont hypothesis"[6,7], in which chloroplasts are assumed to descend from photosynthetic prokaryotes living as endosymbionts within the cytoplasms of heterotrophic protoeukaryotic cells to which they were phylogenetically unrelated, and (2) the "direct filiation" hypothesis[8–10], in which compartmentation of a single photosynthetic cell is assumed to have given rise to the separate and functionally distinct DNA-containing organelles now recognised as nuclei and chloroplasts. With either hypothesis, chloroplasts can be of monophyletic origin (all modern chloroplasts sharing a single chloroplast-like ancestor) or polyphyletic origin (the structurally and biochemically distinct chloroplasts of various major eukaryotic algal groups having multiple and independent choroplast-like ancestors). Determination of how (and how often) chloroplasts arose requires sequence information on molecules for which homologues encoded by the separate genomes of prokaryotes, chloroplasts, and nuclei are known. To date, only rRNAs meet this requirement unambiguously.

Table 1 Oligonucleotide catalogues of blue-green and chloroplast 16S rRNAs

Oligonucleotide sequence	Organism				*Porphyridium*	*Euglena*
	6301	6714	6701	6308		
CC*CCG*	1	1	1	1	1	1
CCCAG	1	1	1	1	1	0
CCACG	0	0	0	1	0	0
ACCCG	1	1	2	1	0	0
CAACG	1	2	1	1	1	1
C*AACG*	1	1	1	1	1	0
ACACG	1	1	1	1	1	0
AACCG	1	0	0	0	0	0
AAACG	1	1	1	1	1	1
AAAAG	0	0	1	1	1	1
UCCCG	1	1	1	1	1	1
CUCCG	1	0	0	0	0	0
CCUAG	0	(0)	0	0	0	1
CUCAG	1	1	1	1	1	1
CCAUG	1	0	0	0	0	0
UCACG	0	1	0	1	0	0
UACCG	1	1	1	1	0	0
CAUCG	0	1	1	1	1	1
UCAAG	1	1	1	1	(1)	2
CUAAG	0	0	?	0	(1)	0
CAAUG	1	1	1	1	1	1
UAACG	1	0	0	0	0	0
UACAG	0	1	0	0	0	0
AUCAG	1	(1)	1	1	1	1
CAUAG	0	0	(0)	0	0	0
ACUAG	1	1	1	1	1	0
AUACG	1	0	0	0	0	0
AAUCG	1	1	1	2	1	1
UAAAG	1-2	1-2	1-2	2	2	2
AAAUG	1	1	1	1	3	3
CCUUG	1	2	1	0	1	1
UUCCG	1	0	0	0	0	0
UUCAG	1	1	1	1	1	0
UCAUG	0	0	1	0	0	0
UAUCG	0	1	1	1	0	0
UUAAG	2	2	1-2	2	2	2-3
UAUAG	0	0	1-2	2	0	0
AUUAG	2	2	2-3	2-3	2-3	2
AAUUG	1	1	1	1-2	1	1
CUUUG	0	0	0	(0)	0	0
UUCUG	0	1	0	0	1	0
UCUUG	0	0	2	2	0	1
UUAUG	0	1	0	0	0	0
CCCCAG	0	0	1	1	0	0
CCAACG	1	0	0	0	0	0
AACCCG	1	0	0	0	0	0
CACAAG	1	1	1	1	1	1
CAAACG	0	0	1	1	0	0
ACAACG	0	1	0	0	0	0
ACAAAG	0	0	1	1	0	0
CCUCAG	0	1	0	0	(1)	0
CUAACG	0	1	0	0	1	0
ACACUG	0	1	1	1	1	0
AACUCG	0	0	(1)	1	0	0
AAUCCG	0	0	(0)	1	0	0
UCAAAG	1	0	1	1	0	1
UAAACG	1	1	1	1	1	0
AAUACG	1	0	0	0	0	1
AAACUG	1	1	1-2	2	0	0
UAAAAG	0	0	1	[illegible]	[illegible]	0
AAUAAG	1	1	0	0	1	1
AAAUAG	0	1	0	0	0	0
UUCCCG	1	1	1	1	1	0
CCUCUG	1	0	0	0	0	0
CUCUAG	1	1	1	0	0	0
CCUUAG	0	1	1	1	0	0
(C$_2$U)AUG†	1	0	0	0	0	0
CUACUG	1	1	0	0	0	0
UACCUG	1	1	0	0	1	0
AUCCUG	1	1	1	1	1	0
UAAUCG	1	1	1	1	1	1
ACUUAG	0	1	0	0	0	0
AAUCUG	1	0	1	1	1	1
AAUUCG	0	1	0	0	1	0
UUAAAG	0	1	0	0	1	1
CUCUUG	0	(1)	1	1	0	0
UCUUAG	0	0	0	1	1	0
UUUACG	0	0	1	1	0	0
AUAUUG	0	1	0	0	1	0
UUUUUG	1	0	0	0	0	0
AACACAG	0	1	(1)	1	0	0
CCUCAAG	0	0	1	0	0	0
CCUACAG	1	0	0	0	0	0
CAACUCG	1	1	(0)	(0)	0	0
UAACACG	0	1	0	0	1	0
CCUAAAG	0	0	0	1	0	0
AACAUCG	0	1	0	0	0	0
AAACUCG	0	0	1	1	1	1
U*AACAAG*	1	(1)	(1)	(1)	1	1
UAUCCCG	0	1	1	1	1	1
CAUACUG	1	0	1	1	0	0
ACUCUAG	0	0	1	1	0	0
AAUCCUG	0	1	1	1	0	0
UAAUACG	1	1	1	1	1	1
CAUUAAG	0	0	0	1	0	0
AUACUAG	0	1	1	1	1	0
AAUACUG	0	1	0	0	0	0
CUCUUCG	1	0	0	0	0	0
CCUUUAG	0	0	0	1	1	0
(UC)CUUAG†	0	0	(1)	1	0	0
UUAUCCG	1	1	1	1	1	1
AUAUCUG	1	0	0	0	0	0
AAUUUUG	0	0	1	0	0	0
UUUUUAG	1	1	0	0	0	0
ACAACCAG	1	0	(0)	(0)	0	0
AA[CA, CCA] G	1	0	(1)	1	1	0
AAACACCG	1	1	0	0	1	0
CCACACUG	1	1	1	1	(1)	1
CAAUACCG	0	1	1	1	1	1
AACACUAG	1	0	0	0	0	0
CCUACAUG	0	(0)	0	0	1	1
CACUCUAG	1	1	0	0	0	0
ACAUCCUG	0	0	1	1	0	0
AAUCUCAG	1	(0)	0	0	?	0
CUCUACUG	0	0	1	1	1	0
UCAUCAUG	1	1	0	0	0	1
UU*ACAAUG*‡	0	0	(1)	(1)	0	0
AAUCAUUG	0	0	1	1	0	0
(CU)UCUUCG †	0	0	0	1	0	0
UUCCUUCG	0	(1)	0	0	0	0
AUUUAUCG	0	1	1	1	0	0
AAUUAUUG	1	1	0	0	0	1
CCCA, CAA, CG	1	0	0	0	0	0
CAACCCUCG	0	1	1	1	0	0
ACAUCCCCG	1	0	0	0	0	0
CUACACACG	(1)	(1)	(1)	(1)	(1)	1
UACACACCG	1	1	1	(1)	1	1
CCUACCAAG	1	0	0	0	0	0
CCUACCAUG	0	(1)	1	(0)	0	0
CUAACUCCG	0	1	1	1	1	0
CCUAUCACG	0	(0)	0	0	0	0
UCAACCAUG	0	0	0	?	?	0
ACUCCUACG	1	(1)	1	(1)	1	0
AUACCCCUG	1	1	1	1	0	0
ACAUCCCUG	0	1	0	0	0	0
(CU$_2$)ACACCG†	0	0	(0)	0	0	0
CUAAUACCG	0	0	1	1	0	0
AAUUCCCAG	0	(1)	0	0	0	0
AUAACACUG	(1)	0	0	0	0	0
CUAAUUCCG	1	0	0	0	0	1
CAUUUCAAG	0	0	0	1	0	0
CAUAUACUG	0	0	1	1	0	0
AAUUCCUAG	0	0	1	(1)	0	0
UUAA, UCAA, G	1	0	0	0	0	0
CAUUUAAAG	0	0	1	0	0	0
C$_{0-1}$,A$_{0-1}$,CUUCUA$_{1-2}$G	0	0	0	?	0	0
UCACUUUCG	0	0	1	0	0	0
CAAUUCUUG	1	0	0	0	0	0
AAUUUUCCG	0	1	1	1	1	1
UUUAAUUCG	1	1	1	1	0	1
UCAAAC$_{1-3}$AG	0	0	0	1	0	0
AAACUCAAAG	(1)	1	1	1	1	1
C$_{0-1}$CCCCUUACG	0	1	1	[illegible]	1	0
UCAC*ACCAUG*	(1)	(1)	(1)	(1)	0	1
UCAAACUCAG	0	0	1	0	0	0
AUAAUACCCG	(1)	0	0	0	0	0
CUCAACUUCG	0	0	1	1	0	0
UACUACAAUG	1	1	1	1	0	0
ACAACAUUUG	0	0	0	1	0	0
CCUAACACAUG	0	1	0	0	0	0
AACCUUACCAG	1	0	0	0	(1)	1
CCAUCAUUCAG	1	0	0	0	0	0
CCAUCAUUAAG	0	1	1	0	1	0
CUUAACACAUG	1	0	(1)	(1)	(1)	1
AAACAUUUAUG	1	0	0	0	0	0
AAU(U$_{1-3}$,C$_{1-3}$)UCUG†	0	0	0	1	0	0
AACCUUACCAAG	0	1	(1)	1	0	0
UUACCCUAACCG	1	0	0	0	0	0
UUACUCUAACCG	0	1	0	0	1	0
UUACCUUAACCG	0	0	1	0	0	0
UUACCUCAACCG	0	0	0	1	0	0
AAU,AU,AU,C$_{2-4}$,AG §	0	0	0	1	0	0
CUAAUACCCAAUG	0	1	0	0	0	0
AACCUACCUUCAG	0	1	0	0	0	0
CUUAACUCCAUAG	0	(1)	0	0	0	0
UACCUAAUAUAAG	0	0	1	(1)	0	0
CCCCUUA, CA, UCCUG	1	0	0	0	0	0
AAAU(UC)CCAAACCG †	1	0	0	0	0	0
CUCAACC(UC)AUACAG†	1	0	0	0	0	0
UAAACUCUUUCCUAG	1	0	0	0	0	0
(C$_{0-2}$U$_{0-1}$A$_x$)AAAAUG†	0	0	(1)	(1)	0	0
UAAA$_{1-2}$C(C$_{1-2}$U)A$_{2-1}$AACUUAG	0	0	1	(1)	0	0
UAAAC,AU,C$_{2-4}$,U$_{4-6}$,AG§	0	1	0	0	0	0
AAAC,AAU,AU,C$_{1-3}$,CCCG§	0	1	0	0	0	0

This table lists all oligonucleotides present in 16S rRNAs of four unicellular blue-green algae and indicates which of these are also present in the 16S chloroplast rRNAs of *Porphyridium*[15] and *Euglena*[17]. Symbols: 1, sequence present (one copy); 2, sequence present (two copies); 1–2, sequence present (one or two copies), 2–3, sequence present (two or three copies); (1), sequence very probably present (one copy); ?, sequence possibly present (one copy); (0), sequence very probably not present; 0, sequence not present. Values of S_{ij} in Table 2 are average values for two comparisons; (*i*) a 'maximum' comparison, for which all sequences designated 1, (1) and ? are assumed present, and possible additional copies of multiply-represented sequences are assumed only when their presence will increase overall coincidence, and (*ii*) a 'minimum' comparison, for which only sequences designated 1 and (1) are assumed present, and possible additional copies of multiply-represented sequences are assumed only when their presence will decrease overall coincidence. Maximum and minimum values of S_{ij} so obtained differ on average by only 1.0 (1–4%) and maximum and minimum values of C_{ij} derived from them (Table 2) would thus differ by no more than 0.5%.

*Post-transcriptionally modified nucleotide.

†Order of nucleotides within parentheses unknown.

‡Modification suggested by unusually migrating secondary digestion product. May be (unmodified) 5′ terminus.

§Relative positions and precise numbers of pyrimidine residues unknown.

Oligonucleotide catalogues

The requisite sequence information can be obtained with techniques developed for bacterial 16S rRNAs[1-3,11-14]. In brief, these involve preparation of high specific activity ^{32}P-labelled 16S (or 18S) rRNA, its complete digestion with ribonuclease T_1 to yield approximately 500 Gp terminated oligonucleotide fragments ranging in length from one to about 15 nucleotide residues, resolution of these fragments by two-dimensional electrophoresis, and determination of the sequence of each oligonucleotide by appropriate secondary and tertiary treatment with nucleases of different specificity. This generates for each rRNA a unique catalogue of oligonucleotides. Enumeration of oligonucleotides of identical (coincident) sequence in catalogues for rRNAs from two different organisms or organelles yields a measure of their relatedness. Some such sequence coincidences of course arise by chance, and the majority of coincidences between dimers, trimers and tetramers will in fact be of this sort. For two completely unrelated (random sequence) RNAs of the size and composition of prokaryotic 16S rRNAs there should, however, arise by chance only about 15 coincidences among pentamers, only about 2 coincidences among hexamers, and, on average, no coincidences among oligonucleotides of greater length[3,15]. Even the most distantly related 16S rRNAs considered here show at least twice this level of sequence similarity and (when oligonucleotides of less than five residues are excluded from the analysis) conclusions of considerable statistical significance can be drawn. For instance, the probability that chance alone accounts for the level of oligonucleotide sequence coincidence observed between the 16S rRNAs of *Synechococcus* 6301 and *Porphyridium* chloroplasts (see below and ref. 15) is approximately 10^{-31}. Table 1 displays recently completed catalogues for the three unicellular blue-green algae described by Stanier[4] as strains 6714, 6701 and 6308 (the last being the Wellesley strain of *Gloeocapsa alpicola*). All three are of what he, on the basis of morphological, physiological and biochemical characters, designates typological group IIA (*Aphanocapsa*)[4]. The GC contents of their DNAs are respectively 47.4, 35.7 and 34.7 mol%. Table 1 also indicates, for each sequence present in one or more of these three strains, its presence or absence in published catalogues of the 16S rRNAs of strain 6301 (ref. 16) (formerly *Anacystis nidulans*), a unicellular blue-green alga of typological group IA (*Synechococcus*) with a DNA GC content of 55.1 mol% (ref. 4); the chloroplasts of a species of *Porphyridium*[15], a unicellular (eukaryotic) marine red alga; and the chloroplasts of *Euglena gracilis*, as recently described by Zablen *et al.*[17].

We can derive from complete data matrices (Table 1) much useful information which will be lost when such data are further reduced to generate measures of similarity (see below). The following are a few of the kinds of comparison which can be made.

(1) There are 39 oligonucleotides (pentamers and larger) which are present in all four blue-green algal strains, and which we here temporarily designate "blue-green invariants". (The number of invariants will certainly fall as more catalogues become available.) Of the 39, 22 are identical to oligonucleotides identified by Woese *et al.*[1] as "universal" among bacterial 16S rRNAs (present in at least 24 of 27 species catalogued), and a further 6 have been described by them as "conserved" (present in at least 14 of 27). Of the 11 blue-green invariants which are neither universal nor conserved in bacteria, two (AUACCCCUG and UACUACAAUG) are readily identified as differing by only a single residue from such sequences (AUACCCUG and CUACAAUG). Conversely, of the 26 oligonucleotides (pentamers and larger) described by Woese *et al.*[1] as universal to bacteria, 22 are blue-green invariants, two (the above-mentioned) differ by a single residue from invariants, and one (CAACUCG) is found unaltered in 6301 and 6714 but altered (AAACUCG) in 6701 and 6308 (and both chloroplasts). Only one (AAUACG) cannot be readily identified in some form in each of the four blue-green species. Thus, most if not all sequences identified (by virtue of their conservatism) as essential to functions of bacterial ribosomes probably retain similar functions in the ribosomes of blue-green algae.

(2) *Porphyridium* chloroplast 16S rRNA contains 64 oligonucleotides (including 32 invariants) which are found in one or more blue-green algal 16S species, while *Euglena* chloroplast 16S contains only 45 such oligonucleotides (including 25 invariants). Twenty-eight of the oligonucleotide sequences shared by one or more blue-green rRNAs and *Porphyridium* chloroplast 16S are found only in this chloroplast rRNA (not also in that of *Euglena*). By contrast, only nine of the sequences shared by blue-green rRNAs and *Euglena* chloroplast 16S are found uniquely in this species (not also in *Porphyridium* chloroplast 16S). Thus the 16S rRNA of *Porphyridium* chloroplasts shows substantially more homology with blue-green algal rRNAs than does the 16S rRNA of *Euglena* chloroplasts (see also below). This was not apparent when the only blue-green algal catalogue available was that of strain 6301 (refs 15 and 17).

(3) Each of the four blue-green 16S rRNAs contains a different variant of the dodecamer UUACXYZAACCG, the variable portion XYZ being CCU for 6301, UCU for 6714, CUU for 6701 and CUC for 6308. It is possible to visualise each sequence as a hairpin with two or three of the variable nucleotides unpaired in the loop and the stem having in three cases four and in one case (6308) three base pairs (including for this purpose one base pair involving the G located 5′ to these sequences in intact 16S rRNA). The 6714 variant of this dodecamer is found also in *Porphyridium* chloroplast 16S rRNA, but no variant of it is detectable in *Euglena* chloroplast 16S rRNAN, nor in any of the bacterial rRNAs described by Woese and his coworkers. We know of no other instance in which such a highly variable region has been found embedded within an otherwise very conservative 16S rRNA oligonucleotide.

Analysis and implications

For taxonomic purposes, the data of Table 1 must be reduced to yield similarity coefficients S_{ij} which measure relatedness in all pairwise comparisons between any two RNAs i and j. One method for doing this is described in the legend to Table 2, and values of S_{ij} computed in this way are shown above the diagonal in that table. Data used include not only the four blue-green algal and two chloroplast 16S rRNA catalogues but, in addition, catalogues obtained by Woese and coworkers for three rather diverse bacterial species. It is possible to derive for each S_{ij} a new value, C_{ij}, which can be interpreted as the probability of any single nucleotide residue having remained unchanged during the evolutionary divergence of rRNAs i and j, or, alternatively, as the percentage sequence homology between these RNAs. Values of C_{ij} (as a percentage) are shown below the diagonal in Table 2.

From Table 2, we see that prokaryotic and chloroplast 16S rRNAs are highly conserved molecules; no pair of them shows less than 80% homology. By contrast, the single cytoplasmic 18S rRNA for which we have appropriate data (that of *Porphyridium*) shows no detectable homology with prokaryotic or chloroplast 16S rRNAs (ref. 15) It shares with *Porphyridium* chloroplast 16S, for instance, no oligonucleotides of more than six residues, and no more than the number of coincident pentamers and hexamers expected to arise by chance. (It should be recognised, however, that any pair of molecules for which C_{ij} is less than about 60% will not show detectable homology by this method.)

Much of the conservatism observed among prokaryotic and chloroplast 16S rRNAs is due to the retention by most of them of sequences designated by Woese *et al.*[1] as universal or conserved among bacteria. There are in fact 18 sequences which are common to all nine 16S species discussed here, and any two RNAs sharing only these 18 sequences would give a value of C_{ij} of about 68%. As a consequence of 16S conservatism, values of C_{ij} vary over a restricted range, and taxonomic inferences must be drawn on the basis of small differences between them Such small differences are significant. For instance, that between 88 and 90% corresponds to a 17% difference in S_{ij}, or to one additional oligonucleotide sequence coincidence for each of the oligonucleotide size classes $N=5$ through $N=10$. Taxonomic distinctions of the type discussed here may, however, perhaps be more readily recognised in comparing values of S_{ij}.

Table 2 Relatedness in pairwise comparisons

Similarity coefficient, S_{ij}

	E. coli	*B. subtilis*	*R. spheroides*	6301	6714	6701	6308	*Porphyridium*	*Euglena*
E. coli		33	37	31	31	28	28	27	27
B. subtilis	86		35	37	34	32	32	34	26
R spheroides	88	87		32	33	30	29	30	23
6301	85	88	86		49	44	41	36	35
6714	85	87	86	92		58	52	47	32
6701	84	86	85	90	94		83	43	33
6308	84	86	84	89	92	98		42	32
Porphyridium	83	87	85	88	91	90	89		36
Euglena	83	82	80	87	86	86	86	87	

% Homology, C_{ij}

There are several ways in which the data of Table 1 can be used to derive single values (similarity coefficients S_{ij}) which measure the relatedness of any pair of RNAs *i* and *j*. That which seems most reasonable to us defines S_{ij} as

$$\left[200\sum_{N=5}^{\infty}k_{N_{ij}}N\right] / \sum_{N=5}^{\infty}(t_{N_i}+t_{N_j})N$$

where N is oligonucleotide length (in residues), $k_{N_{ij}}$ is the number of N-mers common to the two RNAs, and t_{N_i} and t_{N_j} are the total number of N-mers obtained from RNAs *i* and *j*, respectively. S_{ij} is thus equivalent to 200 times the number of individual nucleotide residues present in sequences (pentamers and larger) which are common to two rRNAs divided by the number of individual residues present in all sequences (pentamers and larger) found in both rRNAs. It will range in value from 100 for identical molecules down to 10–15 for totally unrelated species. (Chance sequence coincidences prevent S_{ij} from reaching zero in such cases.) Table 2 shows (above the diagonal) values of S_{ij} computed for the 36 possible pairwise comparisons involving the blue-green and chloroplast catalogues and, in addition, representative bacterial 16S rRNA catalogues obtained by Woese and his collaborators for *Escherichia coli*[13,14], *Bacillus subtilis*[19], and *Rhodopseudomonas spheroides*[20] (a photosynthetic bacterium) For certain purposes it is useful to convert values of S_{ij} to values (C_{ij}) for the probability of any single nucleotide residue having remained unchanged during the evolutionary divergence of RNAs *i* and *j*, since the latter has more obvious physical significance (and can be equated with "% homology"). Such conversion was made with reference to a standard curve whose construction involved the following considerations. The nine rRNAs for which data are presented in Table 2 have nearly the same G content and hence approximately the same number of oligonucleotides of any length N. We define t_N as the average for the nine RNAs of the total number of N-mers and require that two hypothetical RNAs *i* and *j* each have exactly t_N N-mers. If C_{ij} is the probability of conservation of any single nucleotide during the evolutionary divergence of RNAs *i* and *j*, then the likelihood that a given N-mer will similarly be conserved is C_{ij}^{N+1} (the probability that all N residues as well as the preceding G have remained unchanged). Of t_N N-mers originally present, one thus expects on average $t_N C_{ij}^{N+1}$ to have been conserved and $t_N(1-C_{ij}^{N+1})$ to have changed. Of the t_N $(1-C_{ij}^{N+1})$ "new" N-mers which replace them, an average of $t_N^2(1-C_{ij}^{N+1})^2/3^{N-1}$ will by chance have the same sequence as one of the original N-mers which was lost[14] Thus the total number of coincidences observed among N-mers in RNAs *i* and *j* will on average be

$$k_{N_{ij}} = t_N[C_{ij}^{N+1}+t_N(1-C_{ij}^{N+1})^2/3^{N-1}]$$

Using values of $k_{N_{ij}}$ calculated for different values of C_{ij}, a curve relating C_{ij} to S_{ij} for hypothetical RNAs of the sort described was constructed. Values of C_{ij} corresponding to values of S_{ij} shown above the diagonal in Table 2 were read from this curve and are shown (as % homology) below the diagonal.

The blue-green algae taken together are not demonstrably more distant from certain bacterial groups (as represented by *Escherichia coli, Bacillus subtilis* and *Rhodopseudomonas spheroides*) than some of these groups are from each other. For instance, they show on average as much homology (86 8%) with *B. subtilis* as the latter shows with *E. coli* (86%) Although the range of values for S_{ij} and C_{ij} in all pairwise comparisons between individual blue-greens and bacteria is small (28–37 and 84–88%, respectively), each of the four blue-green 16S rRNAs is more closely related to *B. subtilis* 16S than it is to *R spheroides* 16S, and more closely related to the latter than it is to *E. coli* 16S rRNA. The consistency of this pattern supports an earlier suggestion (based only on data for 6301) that blue-greens are more closely related to the bacilli than to the enterics[16]. This relationship extends to post-transcriptional modification patterns; modified oligonucleotides are identical in all four blue-greens and more similar to those of the bacilli than to those of the enterics.

As expected, each of the four blue-green 16S rRNAs is more similar to every other blue-green 16S than it is to any of its bacterial homologues. Strains 6701 and 6308 show most similarity, their homology being as high as that characterising members of the same genus in many bacterial groups (C. R. Woese, personal communication). These two are in turn more closely related to strain 6714 than any of these three is to strain 6301. These findings are in full accord with Stanier's[4] classification of 6701, 6308 and 6714 as members of a typological group (IIA) separate from that containing 6301 (IA)

Porphyridium chloroplast 16S rRNA is unquestionably prokaryotic in nature Data on this molecule were first presented[15] when only one blue-green algal 16S rRNA (that of 6301)[16] had been catalogued. We were at that time puzzled that homology between the two (88%) was not substantially greater than that between chloroplast 16S rRNA and the 16S of a bacterium (*B subtilis* (87%)), and expressed the hope that other blue-green algal 16S rRNAs would show greater specific homology with the chloroplast molecule. Indeed, each of the newly catalogued type IIA blue-green algal 16S rRNAs shows specific high homology with chloroplast 16S Values of S_{ij} or C_{ij} for the three relevant pairwise comparisons are substantially greater than that for any other pairwise comparison involving *Porphyridium* chloroplast 16S (including that with *Euglena* chloroplast 16S) In fact, these values are on average (average $S_{ij}=44$, $C_{ij}=90.0\%$) only slightly below those which characterise the relatedness of the three type IIA strains to 6301 (average $S_{ij}=45$, $C_{ij}=90\ 3\%$). Thus a specifically blue-green algal (rather than generally prokaryotic) ancestor for red algal chloroplasts can be inferred, and this ancestor is likely to have resembled a modern type IIA blue-green more closely than a modern blue-green of type IA.

Euglena chloroplast 16S rRNA is (as Zablen *et al*[17] have shown) also unquestionably prokaryotic, and manifests significantly more homology with each of the four blue-green algal 16S species than with any of the three bacterial rRNAs. The levels and patterns of these homologies are, however, altogether different from those which characterise *Porphyridium* chloroplast 16S. *Euglena* 16S rRNA shows most homology with that of strain 6301, and least with each of those of the three blue-greens of type IIA, the last values being significantly less than comparable values for *Porphyridium* 16S. The level of homology between the two

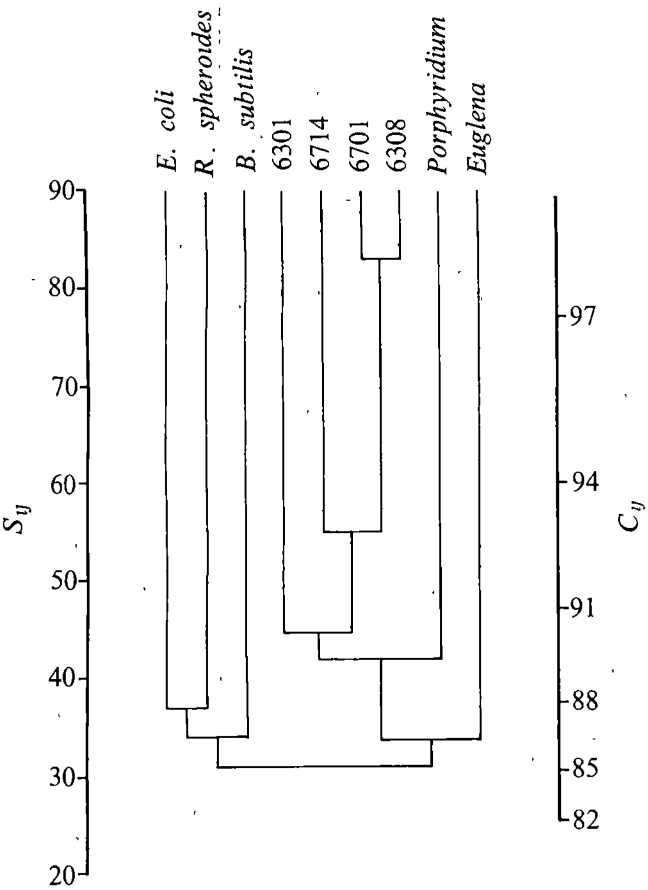

Fig. 1 A dendrogram summarising the relationships between nine prokaryotic and chloroplast 16S rRNAs.

chloroplasts themselves is less than any value of homology involving *Porphyridium* chloroplasts and any blue-green alga. If we take similarity coefficients as representative of evolutionary distance, then we must conclude that *Porphyridium* chloroplasts and blue-greens (at least those of type IIA) share a common ancestor more recent than any shared by *Porphyridium* chloroplasts and *Euglena* chloroplasts. Thus either type IIA blue-greens are "escaped chloroplasts", or, as seems more likely, chloroplasts of the red alga *Porphyridium* and those of the euglenoid *Euglena* arose independently from separate prokaryotic ancestors. Stanier[4,6] has recently emphasised the fact that only the red algae (of all eukaryotic groups) contain chloroplasts which are unarguably of blue-green algal origin. Other chloroplasts may derive from now extinct lines of oxygenic photosynthetic prokaryotes which, had they survived, might not be classified among the blue-green algae.

Figure 1 presents a dendrogram constructed from Table 2, using an unweighted pair-group method (with arithmetic averages)[18]. This dendrogram summarises the data presented here, and is not to be taken as a phylogenetic tree. We would be surprised, however, if the affinities which it indicates between prokaryotic and chloroplast 16S rRNAs were not reflections of true phylogenetic relationships between and among these organisms and organelles.

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Note added in proof: The preliminary catalogue for *B. subtilis* 16S rRNA used in the construction of Table 2 differs by a few oligonucleotides from the catalogue as it will be published[19] (C. R. Woese, personal communication). New values of S_{ij} calculated with this final catalogue differ from those in Table 2 for the pairwise comparisons of *B. subtilis* with *R spheroides,* 6714, 6701, 6308, *Porphyridium* chloroplast and *Euglena* chloroplast, and are respectively: 34, 35, 33, 33, 36 and 27. These minor changes are in no case of sufficient magnitude to alter significantly the conclusions drawn in the text.

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letters to nature

Are supernova explosions driven by magnetic springs?

SUPERNOVA explosions are generally believed[1] to be powered by the gravitational energy of a collapsing core of (Chandrasekhar) mass $\sim 1.4M_\odot$. There is a difficulty, however, with the radial momentum balance: how is the liberated energy converted into the radial motion of an ejected shell? Neither thermal pressure gradients nor neutrino pressures seem to be sufficient to accelerate matter to the observed several per cent of the velocity of light, even after the 1974 rise in the calculated cross section for resonant neutrino scattering. On the other hand, there is increasing evidence that magnetic neutron stars[1-3] form at the centre of (at least a large subclass of) supernovae. I should like to argue that their magnetic pressure is a serious candidate for driving the supernova motion.

According to stellar evolution theory[4,5], a sufficiently massive pre-supernova star is thought to develop a degenerate carbon–oxygen core of mass $\sim 1.4M_\odot$, radius $R_c \gtrsim 10^8$ cm, — matter density $\rho_c \lesssim 2\times10^{10}$ g cm^{-3} which becomes unstable to inverse β decay, and implodes to become a neutron star. The core essentially conserves[6] its angular momentum after formation because molecular friction is negligible, and convective friction is restricted to the shells between chemical discontinuity surfaces; from $\Omega\rho^{-2/3}$=const, and an initial angular velocity $\Omega_i \simeq 10^{-5}\,s^{-1}$ we find $\Omega_c=\Omega_i(\rho_c/\rho_i)^{2/3}\simeq 10\,s^{-1}$. The fast spinning core is likely to be convective[7], and to generate[8] equipartition magnetic fields of strength $B_c=(4\pi\rho_c v_{conv}{}^2)^{1/2}\simeq 3\times 10^9$ gauss. Magnetic flux conservation during core implosion results in neutron star magnetic fields $B_{ns}=B_c(\rho_{ns}/\rho_c)^{2/3}\simeq 4\times 10^{12}$ gauss for $\rho_{ns}\simeq 4\times 10^{14}$ g cm^{-3}, in agreement with observations of pulsars. On the other hand, backward extrapolation[9] of pulsar angular frequencies Ω_p according to $\Omega_p \propto (t+t_1)^{-1/2}$ leads to $\Omega_p\simeq 4\times 10^2\,s^{-1}$ 'at birth' (for $t_1 \sim 3\times 10^2$ yr) in the case of the Crab and the Vela pulsar, a value which is small compared with $\Omega_{ns}=\Omega_c(\rho_{ns}/\rho_c)^{2/3}\simeq 10^4\ s^{-1}$ for a new born neutron star, assuming conservation of angular momentum during core collapse. We infer that a new born neutron star loses angular momentum at a much higher rate than given by the power law above, thereby releasing its rotational energy $\sim MR^2\Omega^2/6=3\times 10^{52}$ erg $\Omega_4{}^2$ on a short time scale ($\sim$ 1 min).

The detailed mechanism may be as follows: conservation of angular momentum during core implosion implies a ratio $E_{rot}/E_{grav}\sim R^3\Omega^2/2GM=\Omega_4{}^2R_{6.1}{}^3$ for $M=1.4M_\odot$, so that gravitational energy E_{grav} would be 100% converted into rotation for $\Omega_4=1$. Note that gravitational radiation cannot remove more than $(GM/Rc^2)^{3/2}(\Omega R/c)^4 \lesssim 10^{-3}$ of the rest energy Mc^2, that is, it does not invalidate our estimate. For $\Omega_4\simeq 1$, only a small fraction of E_{grav} would go into heat and oscillation. The fast rotating collapsed core will not swallow all the initial magnetic flux, and a winding of field lines is unavoidable in the highly conducting surrounding plasma. Magnetic stresses will soon force the plasma into corotation up to the velocity-of-light cylinder of radius $c/\Omega=30\,\mathrm{km}\ \Omega_4{}^{-1}$, and will drive matter towards relativistic speeds beyond it. Winding will increase the magnetic energy density—which temporarily lost significance during core implosion—roughly as $(\Omega t/2\pi)^2$, and equipartition with rotational energy densities will be reached within $< 10^2\ \Omega_4{}^{-2}$ s. In this way, the (huge) rotational energy of the imploded core is tapped by a magnetic spring; cf. ref. 10, where 30 times higher (than ours) initial magnetic field and an assumed axial symmetry led to a jet-like explosion along the rotation axis. Only $\sim 10^{-1}\,\Omega_4{}^2$ of this energy is needed to drive the supernova motion; the rest will heat the plasma by shear viscosity, and field line reconnection.

This generation of magnetic flux by differential rotation will operate only until some volume beyond the velocity-of-light radius has been evacuated, and the ordinary pulsar braking mechanism takes over (magnetic dipole radiation, an electromagnetically driven wind, or magnetospheric friction). This should happen on the same time scale as the build-up of the spring. The newly-born pulsar, having exerted a sizeable torque on the expanding shell, would be expected to have a strong toroidal magnetic field.

In conclusion, it is suggested that magnetic torques between an imploded magnetic core and a surrounding massive shell can explain both the radial momentum balance of supernova explosions, and the small 'observed' spin rates of pulsars at birth.

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Effects of back warming in cocoon stars

THERE now exists general agreement among astronomers that dust shells surrounding young stars are a relatively frequent occurrence, and attempts have been made to calculate some of the properties of such shells (see refs 1 and 2). One obvious possibility is that the dust absorbs the outgoing radiation from the star and re-emits it in the infrared, and this is perhaps the most acceptable explanation for a number of the infrared sources that have been detected in the Orion nebula and in other regions where star formation is suspected to be occurring. If the dust shell absorbs any radiation, then both its inner and outer surfaces will re-emit a certain proportion, though now at a different wavelength, so that some radiation will return to the central star—it is 'warming its own back' so to speak. It is interesting to ask how such a star will evolve when compared with the evolution of a normal pre-main-sequence star. A model for a contracting star which is receiving radiation from an external source has already been developed by the authors[3] in connection with the evolution of Jupiter within the radiation field of the Sun, and here we apply this model to the situation just described.

It should be stressed that we are discussing the evolution of the central star only, the dust being regarded merely as a means of redirecting radiation back on to the surface of this star. We do not tackle the much more complex problem where the dust shell is itself modified because it has modified the star.

We shall also confine ourselves to a purely analytical discussion of the problem. We first need the Eddington[4] plane parallel atmosphere solution modified to the situation where the incoming radiation is non-zero at the boundary, the solution of which has already been obtained by Donnison and Williams[3] as

$$T^4 = \left(\frac{3\tau+2}{4}\right)T_{eff}{}^4 - \frac{I_2}{ac}\left(\frac{3\tau-2}{4}\right) \tag{1}$$

where T is the temperature at optical depth τ, T_{eff} is the effective temperature and I_2 is the specific intensity per steradian moving inwards at the boundary of the atmosphere (that is the contribution from the dust shell). If we let T_E represent the temperature by which the back radiation alone would raise the temperature of the star then we have

$$I_2 = acT_E{}^4 \tag{2}$$

Substituting this expression for I_2 into equation (1) gives

$$T^4 = \left(\frac{3\tau+2}{4}\right)T_{eff}{}^4 - \left(\frac{3\tau-2}{4}\right)T_E{}^4 \tag{3}$$

which reduces to the standard Eddington solution if $T_E = 0$.

For young stars the main source of opacity in the atmosphere is negative hydrogen ions, a convenient form of the opacity law being[5]

$$\kappa = \kappa_0 P^{\frac{1}{2}} T^4 \tag{4}$$

P being the pressure and κ_0 a known constant. The condition of hydrostatic equilibrium in the atmosphere combined with the temperature given in equation (3) yields

$$P^{3/2} = \frac{2g}{\kappa_0}(T_{eff}{}^4 - T_E{}^4)\ln\left[\frac{3\tau}{2}\left(\frac{T_{eff}{}^4 - T_E{}^4}{T_{eff}{}^4 + T_E{}^4}\right)+1\right] \tag{5}$$

g being the surface gravity of the star.

The usual Schwarzschild[6] condition for the onset of convection requires that

$$\frac{T}{P}\frac{\mathrm{d}P}{\mathrm{d}T}=\frac{5}{2} \tag{6}$$

Substitution of the derived expressions for P and T in this equation show that convection sets in at an optical depth τ_c given by

$$\tau_c=\frac{2}{3}\left[\frac{T_{\mathrm{eff}}^4+T_{\mathrm{E}}^4}{T_{\mathrm{eff}}^4-T_{\mathrm{E}}^4}\right](\mathrm{e}^{16/15}-1) \tag{7}$$

Convective stars behave like polytropes of index 3/2 and so the pressure and temperature are known in the interior. At the boundary between the interior and the atmosphere these values must agree with those derived from the atmosphere models. Setting these values equal yields the equation

$$(T_{\mathrm{eff}}^4+T_{\mathrm{E}}^4)^{15}(T_{\mathrm{eff}}^4-T_{\mathrm{E}}^4)^{16}=AM^{28}R^4 \tag{8}$$

A being a known constant.

The luminosity of the central star is given by

$$L=\pi acR^2T_{\mathrm{eff}}^4 \tag{9}$$

and this energy output has to be provided by the gravitational contraction of the star and the incoming energy from the shell. For an object of polytropic index 3/2 this gives

$$L\mathrm{d}t=-\frac{3}{7}\frac{GM^2}{R^2}\mathrm{d}R+\pi acR^2T_{\mathrm{E}}^4\mathrm{d}t \tag{10}$$

The time taken for contraction from an initial radius R_0 is given by substituting for L from equation (9) in equation (10) which yields

$$t=-\frac{3G}{7\pi ac}\int_{R_0}^{R}\frac{M^2\mathrm{d}R}{R^4(T_{\mathrm{eff}}^4-T_{\mathrm{E}}^4)} \tag{11}$$

If we assume that λL is the luminosity which is re-emitted by the shell on to the star then $\lambda=(T_{\mathrm{E}}/T_{\mathrm{eff}})^4$, so that equations (8) and (9) become

$$T_{\mathrm{eff}}^{124}=\frac{AM^{28}R^4(1+\lambda)}{(1-\lambda^2)^{16}} \tag{12}$$

$$L=\frac{\pi acA^{1/3}M^{1/31}R^{66/31}(1+\lambda)^{1/31}}{(1-\lambda^2)^{16/31}} \tag{13}$$

while the integral in equation (11) can be evaluated to give

$$\mathrm{t}=\frac{93GM^{34/31}}{679\pi acA^{1/31}}\left(\frac{1+\lambda}{1-\lambda}\right)^{15/31}\left(\frac{1}{R^{97/31}}\text{ and }\frac{1}{R_0^{97/31}}\right) \tag{14}$$

For a thin shell some of the radiation will pass completely through, so that only part will be absorbed by the shell and re-emitted isotropically. In the limit, when the shell is just thick enough so that no radiation passes through unabsorbed, λ will have the value $\frac{1}{2}$, while for all thinner shells less than half the radiation will return. From equation (12) we can see that for a thin shell the maximum increase in the effective temperature of the central star for a given radius is $[2^{31}/3^{16}]^{1/124}=1.04$, a fairly minimal increase.

Similarly, the luminosity of the star will increase by 1.16, again not a very substantial increase, while the contraction time is also increased by only 1.7. A thin shell will therefore cause no significant change in the structure and evolution of the central star.

For a very thick shell the proportion of radiation returned can be considerably greater than a half, though presumably λ can never actually be unity as some radiation will always be re-emitted outwards. From the observations of possible circumstellar shells (Low *et al.*[7], Wickramasinghe and Nandy[8]) the average emission temperature is ~ 600 K, while presumably their inner surfaces must be limited to maximum temperatures of ~ 3,600 K, the temperature at which all types of grains will melt (Weast[9]). The ratio of radiation escaping from the system to that returning to the star can therefore be as low as $(600/3{,}600)^4$ so that

$$\frac{1-\lambda}{\lambda}\simeq\frac{1}{6^4}$$

If we substitute this value for λ into equations (12) to (14) we find that the luminosity rises dramatically by a factor of ~ 29 and the corresponding evolutionary time by ~ 45. This means that the gravitational contraction now only has to supply energy at ~ 1/45th of the previous rate, so that most of the star's energy comes from what is returned to it by the thick shell. The presence of a thick shell therefore has quite a substantial effect on the star.

Of course, while such a dust shell is there, we cannot see the star, as we have outlined earlier, but instead see the dust shell emitting in the infrared. Once the dust shell clears, however, we see the star in a position and with an age which is considerably different from that which it would have had if it had evolved without back warming from the dust shell.

The effects of back warming by a dust shell will therefore depend very much on the thickness of the shell, with a thin shell having very little effect so that for any shell where some light from the parent central star is observed there will be no significant effect. A thick shell, however, will mask the main characteristics of the star and substantially slow down its evolution.

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Constraints on the nature of the ancient lunar magnetic field

MANY lunar rocks possess a stable component of natural remanent magnetisation (NRM) with specific intensities around 10^{-6} e.m.u. g^{-1} (refs 1–6). Most of them also have compaction ages of more than 3×10^9 yr (refs 5 and 6). Ancient lunar surface magnetic fields of the order of 10^{-2}–1.2 gauss have been postulated to explain the observed NRM in lunar rocks[1–6], and several interesting suggestions have been made concerning the origin of such high magnetic fields during the early history of the Moon. Runcorn and Urey[7] have proposed an intrinsic dipolar field, acquired by the Moon during its initial stages of formation. Such a dipolar field could result either from a primaeval magnetisation of the Moon by an external field or from the action of an ancient lunar dynamo. Collinson *et al.*[5] supported that theory with the observation that the palaeofield required to explain the stable NRM in lunar rocks decreases with the decreasing formation or compaction ages of the rock samples. I point out here some constraints that can be put on the origin and nature of the ancient lunar magnetic field, based on the observation of solar-wind ions in lunar breccias with compaction ages of more than 3.2×10^9 yr.

Considering a lunar dipolar magnetic field, the critical dipole moment required to stop the incident solar-wind ions from reaching the lunar surface is $\sim2.5\times10^{21}$ gauss cm^3, which corresponds to an equatorial surface field strength of $\sim4\times10^{-4}$ gauss. This value has been obtained simply by equating the oppositely directed solar wind dynamic pressure and the magnetic field pressure near the lunar surface. The solar-wind ions are assumed to be incident at right angles to the direction of the lunar dipole axis. The average velocity, v, and number density, n, of solar-wind ions used in this calculation are taken from contemporary solar-wind data as 500 km s^{-1} and 5 cm^{-3}, respectively.

The critical (cutoff) dipole moment and surface field are directly proportional to the product of the velocity and square root of the number density of the solar-wind ions. This dependence is shown in Fig. 1 for a hypothetical range of the parameter $v\sqrt{n}$ which includes the contemporary value; also shown is the predicted lunar surface field 3.2×10^9 yr BP (ref. 5). Though this assignment is somewhat arbitrary when all the available data on lunar magnetism are considered (see ref. 6 for a detailed discussion), the data do indicate the presence of an ancient lunar surface magnetic field greater than a few times 10^{-2} gauss, up to a maximum of 1.2 gauss during the period 3.0–4.1×10^9 yr BP (refs 5 and 6). Thus, it is obvious from Fig. 1 that if the number density and velocity of the solar-wind ions have remained nearly the same throughout the last 4.0×10^9 yr, then the predicted field at the lunar surface more than 3.2×10^9 yr ago is more than sufficient to stop the incident solar-wind ions from reaching the surface.

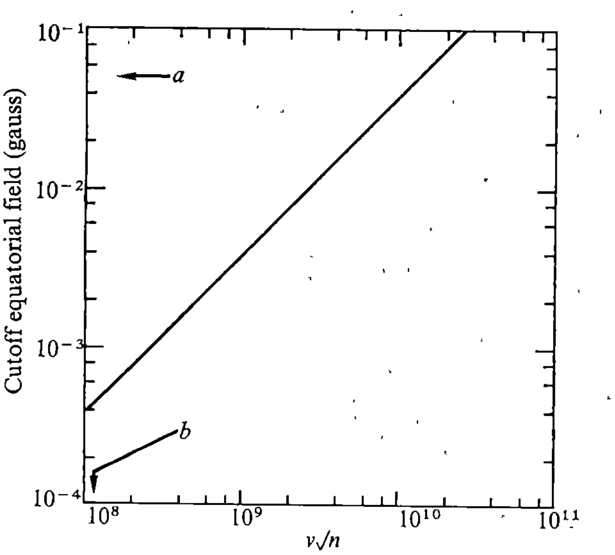

Fig. 1 Cutoff lunar surface magnetic field required to stop incident solar-wind nuclei from reaching the lunar surface, as a function of the parameter v/n, where n is the number density (cm^{-3}) and v the velocity (cm s^{-1}) of the solar-wind ions. *a*, Predicted lunar surface field 3.2×10^9 yr BP (ref. 5); *b*, contemporary solar wind ($n = 5$ cm^{-3}; $v = 5\times10^7$ cm s^{-1}).

These considerations, therefore, imply that solar-wind ions should not be observed in individual components of lunar breccias with compaction ages $\gtrsim3.2\times10^9$ yr. As shown in Table 1, however, there exists at least one lunar breccia (14318) which has an extremely well defined ^{40}Ar–^{39}Ar compaction age of $3.69\pm0.09\times10^9$ yr and is also enriched in solar-wind ion concentration[8,9] (using ^{36}Ar concentration as an indicator of trapped solar-wind ions). In addition, individual components of lunar breccias 14301 and 14313, enriched in solar-wind ion concentration[10], were probably compacted more than 3.2×10^9 yr BP (refs 10 and 11). These experimental observations, therefore, apparently preclude the presence of an intrinsic lunar dipole field during the early history of the Moon, producing a surface field of more than 3×10^{-2} gauss. It is possible, however, that, given certain constraints on the mode of origin and the nature of the ancient lunar magnetic field, both the presence of a large lunar magnetic field more than 3.2×10^9 yr ago, and the observations of solar-wind ions in lunar breccias with compaction ages of more than, or around 3.2×10^9 yr, can be explained.

Table 1 Data from lunar breccias

Sample No.	Type	^{36}Ar (cm^3 STP g^{-1}) $\times10^{-6}$	Compaction age $\times10^9$ yr
14318	Spherule-rich microbreccia	27(9)*	3.69 ± 0 09(8)
14301	Microbreccia with abundant chondrules	61(10)	$\sim$3 7(10,11)
14313	Polymict breccia	360(10)	> 3.2(?)(10)

*Reference numbers in brackets.

Using the model of a global lunar dynamo several alternatives allow the solar-wind ions to reach the lunar surface. First, frequent reversals of the lunar dipole field could occur, producing a long-term field that is nearly zero. These occur in the case of the geomagnetic field, though there is no evidence yet for similar lunar field reversals. Alternatively, a wandering of the lunar dipole axis, could, in principle, allow solar-wind ions to reach different regions of the lunar surface by streaming along the polar direction. Recent analyses of the magnetic fields over the lunar surface indicate that the ancient lunar magnetic dipole axis may not have been oriented along the present rotation axis of the Moon[13]. It is highly questionable, however, whether this evidence by itself can be taken to imply a wandering of the lunar dipole axis. A third remote possibility is a late onset of the dynamo action, such that the individual components of the lunar breccias received their solar wind irradiation before a magnetic field became established. The observed stable component of NRM in lunar samples 62235 and 77017, however, requires that the onset of the lunar magnetic field must have taken place not later than 4.1×10^9 yr BP (ref. 6).

Models invoking external field magnetisation as the cause of the ancient lunar magnetic fields constrain the dipole axis along the rotation axis, so field reversals are not possible. Further, because of the large magnitude of the external field ($\sim$20 gauss) required by such models, it has been proposed[7] that such conditions can only have been fulfilled during the very early stages of the Solar System, more than 4.5×10^9 yr BP.

Finally, considering a local field model[12], none of the constraints required in the global dynamo model are necessary to explain the observation of solar-wind ions in old lunar breccias. Since the trajectories of the solar-wind ions in a local magnetic field depend on the magnitude, scale length and direction of the field[14], there always exists the possibility that the solar-wind ions may reach the lunar surface. Further, the absence of a perfect correlation between the inferred magnetic field strengths at the lunar surface and the compaction or formation ages of the rock samples[5,6] can also be explained within the framework of the local field hypothesis. The greatest drawback of the local field hypothesis is the absence of a precise model concerning its origin. One of the models proposing the presence of 'fescons', small pockets of Fe–FeS

It is implicit in this discussion that the physical properties of the solar-wind ions have remained the same throughout the last 4×10^9 yr or more. As noted already, the critical surface field strength of the lunar magnetic field required to stop the incident solar-wind ions from reaching the lunar surface is directly proportional to the parameter $v\sqrt{n}$. Therefore, if the value of this parameter was at least two orders of magnitude higher 3.2×10^9 yr ago than today, the solar-wind ions could still have reached the lunar surface even if the lunar surface magnetic fields were $\sim3\times10^{-2}$ gauss (see Fig. 1). Assuming that the velocity of the solar-wind ions has remained the same, such an increase in $v\sqrt{n}$ implies an increase of more than four orders of magnitude in the number density of the solar-wind ions over the contemporary value. Although a case can be made for an enhanced solar wind flux during the very early active phase of solar evolution, close to 4.6×10^9 yr BP, it is highly unlikely that this will be the case at around 4.0×10^9 yr BP or later, times relevant to this discussion. Higher orders of magnitude cannot be advocated for the velocity of the solar-wind ions for a similar reason. Further, rare gas analyses of the gas-rich achondrite Khor Temiki have conclusively shown[15] that the range of solar-wind ions in silicate minerals of this meteorite is $\lesssim$2,000 Å, corresponding to an energy of $\sim$ 1 keV/a.m.u. Since the solar wind irradiation of the Khor Temiki meteorite predated its compaction age, which is $> 4.0\times10^9$ yr, as indicated from preliminary data (D. Leich, personal communication), it can be safely concluded that the velocity of the solar-wind ions has remained roughly the same during the past 4×10^9 yr.

In conclusion, observations of solar-wind ions in individual components of lunar breccias with compaction ages of more than 3.2×10^9 yr rules out the hypothesis that external field magnetisation initiated the ancient lunar magnetic field. This hypothesis is also unsuitable for other reasons concerning lunar magnetism[5,13]. The lunar dynamo model is a possible alternative only if an *ad hoc* assumption like field reversal is introduced. A strong case cannot be made for the local dynamo model[12] as it is yet to be fully developed both from an analytical and an experimental viewpoint.

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Longitudinal dependence of ionospheric total electron content in the South Atlantic Geomagnetic Anomaly

WE present here a report on the changes with longitude of the total ionospheric electron content (TEC) found in the region known as the South Atlantic Geomagnetic Anomaly (SAGA). These have been investigated by measurements of the phase delays of the modulation of transmissions from the ATS-6 radio beacon[1,2]. The ATS-6 geostationary satellite moved from a position of 94°W to 34°E, between May 23 and June 21, 1975, thus providing the opportunity to investigate this longitude dependence.

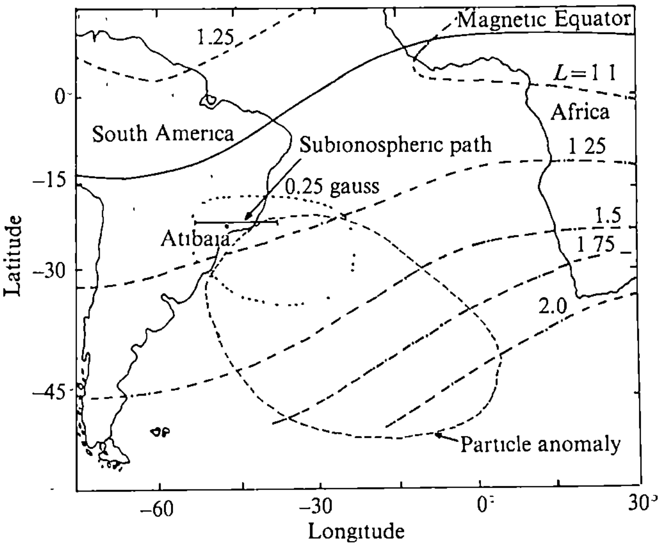


Fig. 1 Simplified map of the South Atlantic anomalous area. It shows the satellite's 420-km altitude subionospheric path, the 0.25-gauss magnetic contour at the Earth's surface, the *L* parameter calculated for 500 km altitude, and the contour for the 'particle South Atlantic Anomaly' after Ginzburg *et al.*[13].

Our method of determining the TEC, based on the group delay technique, is insensitive to the values of the geomagnetic field, for the v.h.f. region[3,4] (140 MHz and 360 MHz, with a 1-MHz modulation frequency), and the electron content includes the ionosphere and the plasmasphere. The TEC can be determined from the simplified equation

$$\int N \mathrm{d}s = 2.7\times10^{16}\Delta\varphi \ \mathrm{m}^{-2}$$

which is derived from well known formulations[1,5], s (m) is the ray path from the satellite to the receiving station, N (m^{-3}) is the electron concentration, and $\Delta\varphi$ (rad) is the measured group delay. The reported TEC values refer to $\int N \mathrm{d}h$, where $h = s/\sec\chi$, and χ is the satellite direction's zenith angle at the subionospheric point situated at an altitude of 420 km.

ATS-6 transmissions were picked up with two circularly polarised antennae at Itapetinga Radio Observatory, Atibaia, São Paulo, Brasil (latitude −23°11′03″, longitude 46°33′48″ W). The satellite was tracked from 94°W to 7°E, corresponding to a 420-km altitude subionospheric path of 53°W to 40°W, at a latitude of $\sim$ −21°, as shown in Fig. 1. Observations beginning May 3, 1975, were also carried out for several days before the satellite's movement. With the exception of May 19–20, for which some geomagnetic activity was reported, the entire period covered in this experiment was extremely quiet, both magnetically and in terms of solar activity[6]. In Fig. 2 we show the superimposed TEC diurnal values reduced to the subionospheric point vertical ($\int N \mathrm{d}h$), before the satellite movement (*a*), and over the days of satellite movement (*b*). Higher values were obtained before the satellite movement, at a subionospheric longitude of 53°W. Averages and s.e.m.s for this longitude were, at maximum (just after local noon 17–18 UT) $(29.6\pm1.3)\times10^{16}$ m^{-2}, and at minimum (the predawn hours from 6–7 UT) $(4.7\pm0.7)\times10^{16}$ m^{-2}. These values are comparable with values obtained at Nairobi, near the Equator, but outside SAGA, during quiet Sun conditions, by the Joint Satellite Studies Group[7], using the Faraday rotation technique for TEC determinations.

In Fig. 3 we indicate the TEC values as a function of the subionospheric longitude where the transmissions were received.

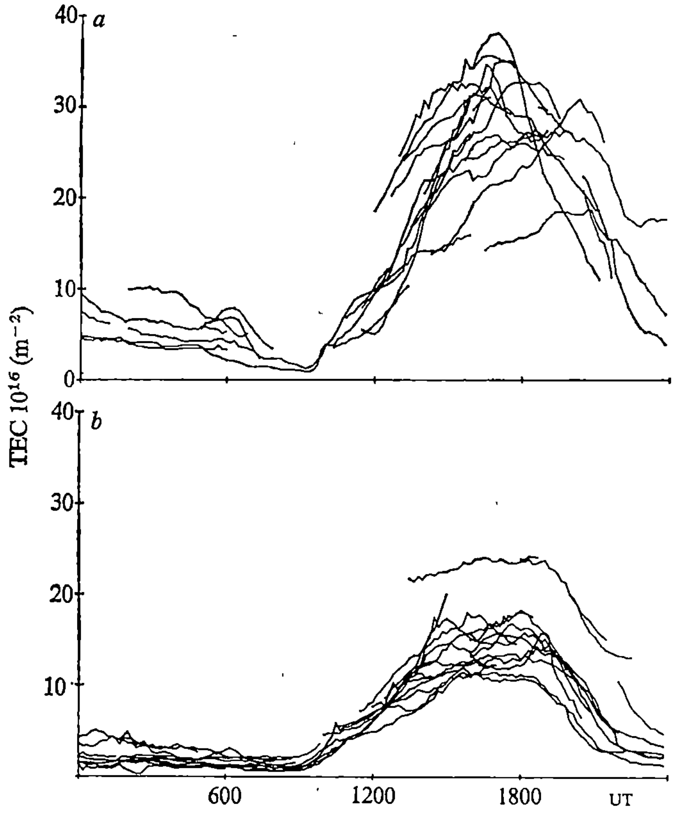
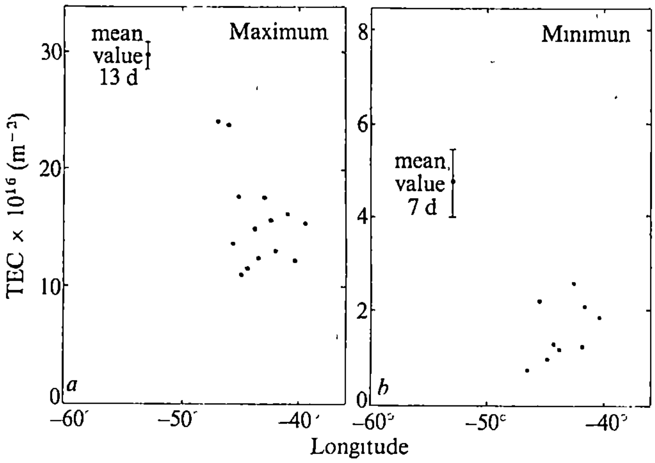


Fig. 2 Daily plots of the total electron content (TEC) in UT (LT is UT$-$3 h). *a*, For the days before the satellite movement—a subionospheric longitude of 53°W. *b*, The days when the satellite was moving east. The two abnormally high values in (*b*) come from satellite positions before 46°W, as shown in Fig. 3*a*.

The first value, at 53°W, corresponds to the average of the period previous to the satellite movement. The transmissions were very poor during the first days of movement, and useful information was obtained only from 53° to 47°W. A very substantial reduction of the TEC values was then obtained in the longitude range of 46°W–40°W, both at daytime maximum (Fig. 3*a*) and at pre-dawn minimum (Fig. 3*b*). Average values for the 46°W–40°W longitude range were of $(14.1\pm0.7)\times10^{16}$ m^{-2} at maximum (17–18 UT) and of $(1.7\pm0.2)\times10^{16}$ m^{-2} at minimum (6–7 UT), corresponding to reductions in TEC of 52% and 64% compared with the 53°W figures. In Fig. 3*a* there is still a suggestion of a sharp transition in TEC at ~ 46°W.

Fig. 3 Longitude dependence of TEC at maximum *a*, and at pre-dawn minimum, *b*, Significant decreases were found when the satellite was eastwards of ~46°W. Note that the first point at 53°W is an average for the days before movement, and the bar is the s.e.m.



The observed change in the TEC cannot be attributed to a seasonal effect, since the entire period of observation covered only 44 d. If we take typical equinox-to-winter midday reduction in TEC, in conditions of small sunspot number[8], we find that in the period covered by our experiment, the seasonal influence on the TEC reduction should be of about only 6%. On the other hand, we do not think the observed changes in TEC could be attributed to the dependence of electron concentration on the magnetic dip angle effect[9,10]. The variation of longitude from the subionospheric point corresponded to a change in dip angle from $-18°$ to $-26°$ (ref. 11), too small to produce a significant change in the TEC.

All the 420-km altitude subionospheric path observed is situated within a 0.25-gauss magnetic contour (at the Earth's surface), corresponding to the so-called Brazilian Geomagnetic Anomaly. Our results suggest that the effect on TEC is displaced eastwards from that region. This is consistent with Roederer's[12] interpretation of the effect produced by the eccentric Earth magnetic dipole position, displacing the trapped particle shell eastwards over the Atlantic Ocean. This 'particle anomaly' has been described, for example, by Ginzburg *et al.*[13]. It shows higher fluxes of charged particles, as measured by satellite counters in the altitude range 187–339 km (as was also indicated in Fig. 1). In a more recent study Torr *et al.*[14] have shown that in this area there are increased precipitated electron and proton fluxes. They find a sharp rise in the proton flux for an L shell value of 1.5 at ~ 30°W, and in the electron flux at $L=2$ at about the same latitude.

Interpretation of aeronomic phenomena in SAGA is difficult and often controversial[15,16]. Reduction in electron concentration in the Anomaly has been already suggested by Torr and Torr[17], as a net result from excess corpuscular bombardment of the upper ionosphere, producing additional ionisation, and also temperature increases, increasing the scale height and the loss rates. Indeed, Taylor[18] has shown that for an F-region trough (that we might take as a similar effect in a short time interval) a significant reduction of electron concentration occurred while increases in the electron and ion temperatures were observed.

One possible explanation for the decrease of the TEC in the SAGA is that the velocity terms in the continuity equation[19,20] for electrons are important

$$\frac{1}{N}\frac{\partial N}{\partial t}=-\frac{1}{N}\mathbf{V}\nabla N-\nabla\mathbf{V}$$

where $\mathbf{V}=(\mathbf{E}\times\mathbf{B})/B^2$ is the electromagnetic drift velocity that arises from the magnetospheric dawn-to-dusk large scale electric field $\mathbf{E}$, which can have an important effect on the motion of plasmasphere low energy charged particles[21]. The total divergence $\nabla\cdot\mathbf{V}$ is usually negative[20], although it may depend on assumptions made about the motion $\mathbf{V}$ when the ion drag effect is important[19]. Thus the proposed explanation could be tenable only if the first term on the RHS of the above equation is positive and sufficiently large. This may be true within the SAGA as a result of inherent gradients of N. Further quantitative researches are suggested for the establishment of an adequate model for explaining the observed TEC reductions in the Anomaly.

Diurnal TEC measurements of Faraday rotation taken in the same period confirm diurnal change reduction as the satellite moved eastwards. Another study relating the two methods of TEC determination is in preparation.

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Hudson Bay, Lake Zissaga and the growth of the Laurentide Ice Sheet

THE Laurentide Ice Sheet, by far the largest of the Northern Hemisphere continental glaciers, has formed and disappeared several times during the Quaternary[1]. I suggest here that, at the times of its formation and early growth, the mass balance of the ice sheet depends strongly on the physical behaviour of Hudson Bay, which is in turn largely controlled by the growing ice. Rapid changes in the size, salinity, and ice and snow cover of Hudson Bay must all have important effects on the mass balance of the growing Laurentide Ice Sheet.

Ives *et al.*[1] have advocated the concept of 'instantaneous glacierisation', whereby continental glaciation in the Northern Hemisphere is initiated by the appearance of perennial snowfields on Baffin Island and in northern Labrador, and they present considerable field evidence in support of their views. I have taken the same position on theoretical grounds[2]; a region with a high albedo absorbs relatively little energy from the Sun, but constantly loses energy as outgoing infrared radiation. It thus has an energy deficit that can only be balanced with advected energy from the surrounding area. If a significant fraction of the energy available for advection is in the form of latent heat, and if the region is cool enough so that precipitation generally freezes, more snow will fall each year than melts, and the snowfield will grow into an ice sheet.

Because of its position, Hudson Bay must be an important source of moisture for the formation of perennial snowfields on Baffin Island and in northern Labrador and for their initial growth; at present, snow falls during all seasons, and the highest snowfall occurs during autumn and early winter, before Hudson Bay freezes[3,4]. Once ice sheets form and begin to grow, the sea level will fall, however, and this will decrease the size of Hudson Bay and its effectiveness as a moisture source. This negative feedback mechanism will retard or reverse the growth of the ice sheets, leading either to steady-state ice sheets or perhaps to systematic oscillations in ice sheet size.

This feedback relationship will operate only while Hudson Bay is an arm of the ocean. A growing ice sheet on Baffin Island will, however, eventually form an ice dam that will block Hudson Strait, as a study by Mahaffy[5–7] has suggested. This would transform Hudson Bay into an immense proglacial lake and alter its size, salinity, and direction of drainage. This lake is designated here as Lake Zissaga (from an Indian word for the onset of winter).

With Hudson Strait blocked by ice, Lake Zissaga will increase in size and decrease in salinity until it finds an outlet, flooding low lying land and eventually draining to the south through the Great Lakes. These changes will act in two ways to intensify the developing glaciation, depending on whether Lake Zissaga is frozen or not.

If the lake is not frozen, its increased size will make it a more effective moisture source for the developing ice sheet, and this will lead to renewed or augmented growth of the ice sheet kernels on Baffin Island and in northern Labrador, which can draw moisture from the lake to help balance their local energy budgets using latent heat.

If Lake Zissaga is frozen, it will no longer be an effective moisture source for the ice sheet kernels in the north, but it will have a high albedo and a large deficit in its local energy budget, which, as noted above, must be balanced using energy advected from the surroundings. If this advected energy includes large amounts of latent heat and therefore produces high snowfall, Lake Zissaga will become permanently ice covered and will become a new centre of growth for the Laurentide Ice Sheet.

There are several reasons why the permanent freezing of Lake Zissaga is not unlikely. Oceanographic data, although sparse, suggest that Hudson Bay now has an energy deficit of a few percent that is balanced either by movement of relatively warm water into Hudson Bay from the Atlantic, or by a net export of ice[8]. Neither of these mechanisms would operate for Lake Zissaga. Freezing would also be aided by the low salinity of the lake, as fresh water both freezes and melts at higher temperatures than salt water[9]. This would cause Lake Zissaga to remain frozen for a longer period each year than Hudson Bay does, and would increase the regional energy deficit because of the effect of the ice on the albedo. Yet another effect is that the inflow of warm water from the surrounding land areas would be reduced, because of the flooding of low areas and the consequent reduction in land area draining into the lake For all of these reasons, it seems likely that Lake Zissaga, once it formed, would have a strong tendency to freeze over and stay frozen.

Permanent freezing of Lake Zissaga will result in a sudden southward shift in the area of maximum snow accumulation on the growing Laurentide Ice Sheet. It will extend the area with a large energy deficit much closer to the Gulf of Mexico, which is an important source of warm, moist air masses that nourish the growing ice sheet while balancing its regional energy budget. This southward shift will significantly change the relationship between the growing ice sheet and the oceans from which it derives both moisture and energy. The magnitude of the total energy deficit over the ice sheet will increase considerably, and at the same time the energy gradient between the ice sheet and the oceans will intensify, both because of the increased total deficit and because of the decreased distance between them. These changes will increase the rate of accumulation of snow on the ice sheet, and they may also alter the oscillation period between stadial and interstadial periods.

Another possibility is that the ice dam may be somewhat unstable during its early phases, so that the accumulation of water in Lake Zissaga could cause the dam to break before the lake freezes to the bottom. If the ice cover were floating at the time the dam broke, much of the ice could be swept away during the draining of the lake; this would restore a low surface albedo and eliminate the local energy deficit required to drive a continental glaciation. Such a mechanism provides a plausible, although not yet proven, way to account for the short but dramatic global coolings that Flohn[10] has termed "abortive glaciations".

The concept of 'instantaneous glacierisation' may thus be extended to include not only the initial formation of ice sheet kernels on Baffin Island and in northern Labrador, but also the freezing of Lake Zissaga and its sudden transformation from a large proglacial lake into a large, thin, growing ice sheet.

If the Laurentide Ice Sheet originated in the North, as Ives and his coworkers have suggested[1], then Lake Zissaga must have existed, and it must have been an important factor in the development and growth of the Laurentide ice. The episodic nature of Northern Hemisphere continental glaciation during the Quaternary must be in part a response to the huge perturbations to the global climatic system introduced by the permanent freezing of Lake Zissaga.

If all of this is true, then local geographical conditions may allow small ice sheets such as those that grew on Baffin Island to produce significant environmental changes far beyond their own margins, and these changes must be understood before we can reach a complete understanding of the dynamics of ice ages.

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Earth tides, volcanos and climatic change

A STATISTICALLY significant correlation has been found between the derivatives of the envelopes of peak tidal stresses at high northern latitudes and the mean temperature of the Northern Hemisphere as reflected in oxygen isotope ratios in the Greenland ice cap. We suggest that variations in tidal stresses on the Earth caused by the Sun and Moon cause changes in the amount of stratospheric dust produced by volcanic activity; this in turn changes the thickness of the stratospheric dust veil and hence the atmospheric radiation balance. We find that, for a simple model, periodic variations in tidal stress account for 13% of the variance in the ice-core temperature record.

The connection between volcanic activity and the stratospheric dust veil is well documented[1–3], and a resultant effect on mean global and hemispheric temperatures has been demonstrated[2–4]. Thus, an ability to predict volcanic activity would certainly help in predicting climatic changes. We have therefore examined the hypothesis that volcanic activity is triggered by quasi-periodic increases in the amplitudes of the tidal stresses exerted on the Earth.

Tidal forces produce stresses in the Earth's interior and crust. Because of the Earth's rotation and the Moon's orbital motion, any particular place on the Earth's surface experiences two complete cycles of high and low tidal stress roughly every 25 h. The Earth and Moon follow elliptical orbits whose planes are tilted with respect to each other, and whose orbital elements are changing continuously (particularly those of the Moon). Thus, the amplitude of the basic 12.5-h tidal stress cycle follows complex but completely predictable patterns[5,6].

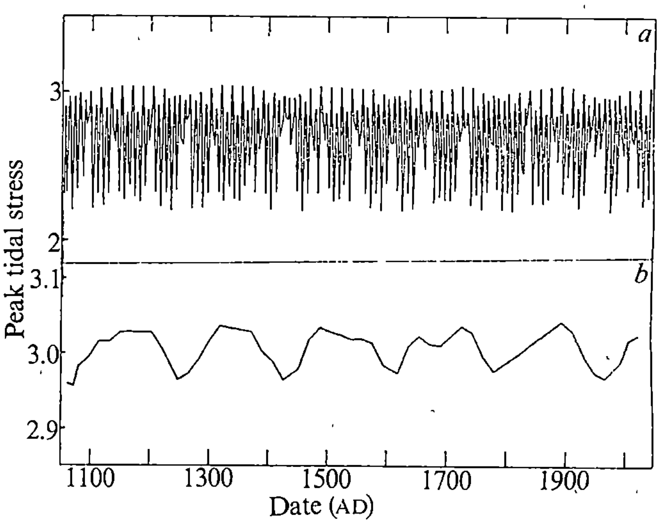


Fig. 1 *a*, Upper envelope of fortnightly tidal stress peaks at 60°N latitude. One unit on the vertical axis represents 4.532×10^{-5} N kg^{-1}; *b*, Upper envelope of Fig. 1*a* with 8.85- and 18 61-yr periods filtered out. Note change in vertical scale.

Correlations have been reported between the fortnightly tidal stress peaks caused by the Moon's orbital motion about the Earth and such events as eruptions of Mt Stromboli[7] and Yellowstone Park geysers[8]. Tectonic activity might therefore be expected to correlate with a tidal period of $P_1 = 8.85$ yr caused by the advance of the longitude of the Moon's perigee ($\tilde{\omega}$) (which determines the times of alignment of the Moon's perigee with the Earth's perihelion) and with the period $P_2 = 18.61$ yr of the regression of the longitude of the node (Ω) of the lunar orbit on the ecliptic (which affects the exactness of the alignment of the Moon's perigee with the Earth's perihelion). There is a commensurability between these two angles, in that the argument

$$\theta = \tilde{\omega} - 2\Omega,$$

has a long period variation, P_r, given by

$$1/P_r = 1/P_1 - 2/P_2$$

(minus because the node regresses). The value of P_r from this relation is 179.3 yr, suggesting there should be a long term tidal resonance effect of this period.

Precise values for these periods are as follows, where T is in tropical centuries (36,524.22 d) from 1900.0

$$P_1 = 8.84750 + 0.0000225T$$
$$P_2 = 18.61330 + 0.0000200T$$
$$P_r = 179.33134 + 0.005511T$$

Thus, the resonance period was 179.29276 yr in AD 1200 and is 179.33553 yr in AD 1976.

To examine in detail the nature of the effects of this long period in the tidal stress produced on the Earth, we first calculated the semi-monthly maximum stresses over the period AD 1200–1970 at latitude 60°N (the region of greatest northern hemispheric volcanic activity)[3,9]. The tidal terms for the Moon were expanded to second order in the Earth's radius, and those for the Sun were expanded only to first order (Fig. 1*a*). We then filtered out the 8.85- and 18.61-yr periods by taking suitable envelopes (Fig. 1*b*). The first derivative with respect to time of this filtered record of the peak tidal stress then gives a measure of the average rate of change of long period stress effects in the Earth's core and crust, as well as the rate of change of the amplitude of any resultant strain, flexure, friction, or other results of stress.

Figure 2 shows the power spectrum of the first derivative of the peak tidal stress for latitude 60°N. Also shown is the power spectrum calculated by Dansgaard and coworkers[10] for temperature changes indicated by variations in the oxygen isotope ratio ($\delta^{18}O$) record in the Camp Century, Greenland ice core (also for the time span AD 1200–1970). We choose this climatic record because of its consistency, accuracy and time resolution and (perhaps because of its location at high latitude inside the circumpolar vortex) because it appears to be an exaggerated representation of the mean mid-latitude Northern Hemisphere temperature. Peaks with periods near 180 yr appear in both the $\delta^{18}O$ and 60°N tidal stress derivative curves. Mitchell (personal communication) has pointed out that although the two highest peaks in the $\delta^{18}O$ power spectrum (180 and 78 yr ± 5%) can be considered as "statistically significant by the conventional criterion (*a priori* test), they were not statistically significant on the basis of *a posteriori* tests in which the location of these two peaks in the spectrum was not predicted in advance from independent information".

The correlation coefficient between the derivative of tidal stress at 60°N and the $\delta^{18}O$ temperature curve is 0.37; the probability that no true correlation exists as determined by the Student's t test is $\leqslant 0.005$. The square of the correlation coefficient tells us what fraction of the Greenland ice curve can be 'explained' by the tidal force curve; thus we can ascribe 13% of the variance in the $\delta^{18}O$ curve to derivative tidal stresses at 60°N. We have also calculated tidal stress derivatives for 30°N and find a strong 179.3-yr periodicity there too, but this periodicity was not found at the Equator.

Figure 3 shows a time series comparison between the $\delta^{18}O$ temperature curve (smoothed by running means) and the calculated tidal stress derivative at 60°N. In spite of the positive correlation of the two curves, two epochs seem to be anti-correlated (~1420–1530 and 1650–1700). This discrepancy may be caused by the action of climatological variables associated with changes in solar activity, since the two discrepant epochs found here are nearly centred on the two strongest solar activity minima over the entire 770-yr period[11].

Although we have found a statistically significant correlation between tidal forces acting on the Earth and the mean temperature of the Northern Hemisphere, demonstration of a deterministic connection between tidal stresses and temperature requires an understanding of the evolution of the stratospheric dust veil as well as the causes of volcanic activity. Although the stratospheric dust veil receives sensible contributions of volcanic dust from explosive volcanic eruptions[1], it seems that the main contributor is sulphur dioxide gas (SO_2) vented from many uncatalogued sources as well as the hundreds of known 'active' volcanos (J. B. Pollack, O. B. Toon, C. Sagan, A. Summers, W. Van Camp, and B. Baldwin, unpublished). This gas reacts in the stratosphere to produce sulphur-bearing aerosols as well as sulphuric acid coatings that increase the optical effects of sub-micron nuclei[12]. A thicker sulphurous dust veil reflects more sunlight into space and cools the Earth's surface (ref. 4, and J. B. Pollack *et al.*, unpublished).

Volcanic activity serves as a mechanism to release thermal energy from the Earth's interior. Thus, we can view the Earth as a boiler and the inactive volcano or vent as a sealed valve. Conversion of tidal energy to thermal energy by friction is concentrated at plate boundaries[13], where almost all active volcanos are found. Thus tidal energy helps heat up the boilers and increase the pressure, while tidal stresses weaken and break the seals. Both of these triggering effects increase during periods of increasing peak tidal stress.

Heaton[14] has shown that shallow, larger magnitude earthquakes tend to be triggered by tidal stresses and Tokarev[15] has found that such earthquakes precede the eruptions of andesitic and dacitic volcanos and outbursts of adventive craters. Thus there seems to be firm support for the belief that tidal forces can trigger volcanic eruptions.

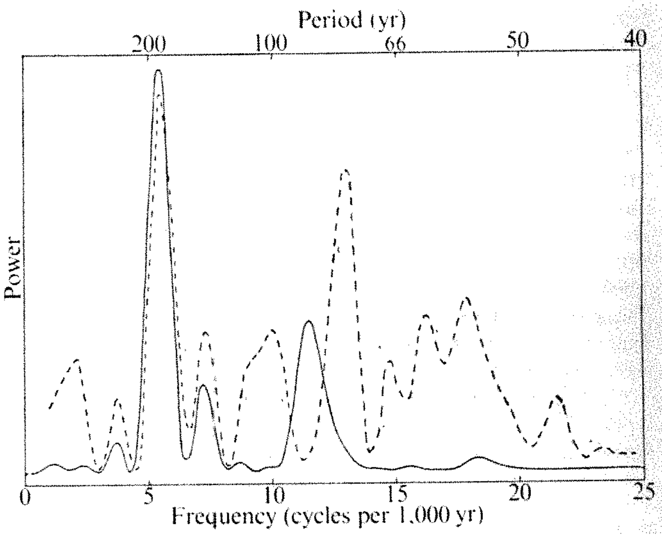
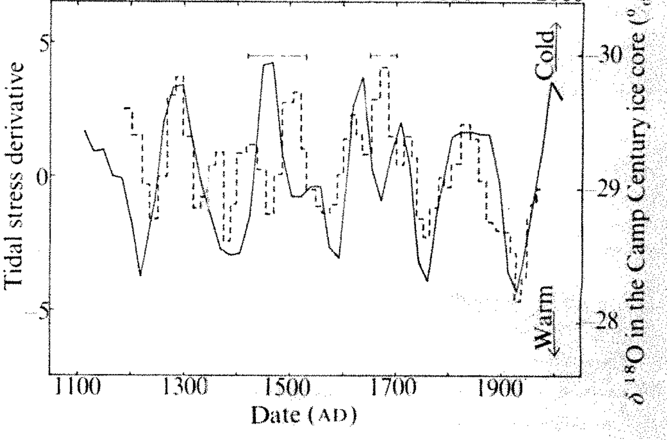


Fig. 2 Power spectra of the $\delta^{18}O$ Greenland ice temperature curve[10] (— — — —) and the derivative of the envelope of the peak vector sum tidal stress at 60°N latitude (——) for the period AD 1200–1970. The peak in the tidal stress derivative at 179.3 yr arises from variations in the Moon's orbital parameters.

Tokarev has also found that the growth of cumulative seismic strain release before andesitic volcanic eruptions generally follows a hyperbolic curve on a time scale of weeks to months. Although the time scales involved in our work are much longer, we hypothesise that a similar trend is effective in triggering activity in the assemblage of climatically important volcanos. In other words, once a volcano has erupted, its susceptibility to triggering remains low for a longer period of time and then increases rapidly following a hyperbolic or exponential curve. Thus the available stress can exceed the stress required for triggering during periods when the available stress is not decreasing rapidly. Therefore, tidal stress triggering of eruptions occurs preferentially when the value of the derivative is increasing. Since our argument is a statistical one, we are not prepared to speculate on the details of the geophysical mechanism(s) involved.

Even though a statistical relationship has been found between tidal stresses and climatic change, there are ample reasons to

Fig. 3 Derivatives of the envelope of the peak vector sum tidal stress at 60°N (———) and three-point running means of the $\delta^{18}O$ Greenland ice temperature curve (— — — — —). Periods of minimal solar activity[11] are marked with horizontal bars. One unit on the vertical axis equals 4.532×10^{-5} N kg^{-1} d^{-1}.



expect differences between the $\delta^{18}O$ curve and the tidal stress curve. They include:

(1) The temperature curve used here is only a proxy record of what the instrumentally determined mid-latitude temperature would be. Various proxy records disagree[3].

(2) This analysis uses tidal force calculations from only one latitude. A more complete investigation would include integration over all latitude belts with high resolution near the Equator and weighting determined from measured dust and SO_2 emission rates.

(3) The correlation technique used here implicitly assumes a linear relationship between temperature and tidal stress. The true relationship certainly must take some more complex functional form.

(4) Other physical causes for climatic change are also at work. In particular, climatic influences ascribed to solar variability have been widely discussed[16].

(5) Feedback effects are not considered here. They may be very important over fairly long time scales[17].

We have presented evidence that at least 13% of the variance in the $\delta^{18}O$ temperature record in the Greenland ice cap might be ascribed to periodic variations in tidal stress. We suggest that the 180-yr periodicity found in the $\delta^{18}O$ record is caused by regular changes in the Moon's orbital motion, with tidal stresses as the physical link. We believe that this mechanism acts along with variations in solar and geomagnetic activity[11,16], stratospheric ozone (R. J. Angione, E. Mederios and R. G. Roosen, unpublished) and other as yet undiscovered physical mechanisms to produce deterministic and predictable climatic changes.

The search for physical mechanism by means of correlation analyses is complicated by the fact that the Universe is filled with periodic and quasi-periodic phenomena, many of which might be affecting the Earth's climate. For instance, 180-yr periodicities in solar activity have been suggested by both the planetary theory of sunspots[18] and natural oscillations inside the Sun (C. L. Wolff, unpublished). It is not clear whether this 'coincidence' is fortuitous or whether two or more separate physical mechanisms are acting in concert.

As a test of our tidal force theory we offer a prediction. The 179.3-yr periodicity in tidal stress at high northern latitudes is precisely out of phase with that at high southern latitudes. Since it is generally believed that stratospheric dust from volcanos outside the tropical ozone is not efficiently transported from one hemisphere to the other, we expect that any 179.3-yr cycle found in Antarctic ice cores should be exactly out of phase with that found in the Greenland ice core. This prediction is of course endangered by the comparative sparsity of active volcanos at high southern latitudes[9].

Since accurate prediction requires that virtually all of the variance be explained, we do not claim here to have achieved a meaningful climatic prediction, but we are encouraged by these preliminary results.

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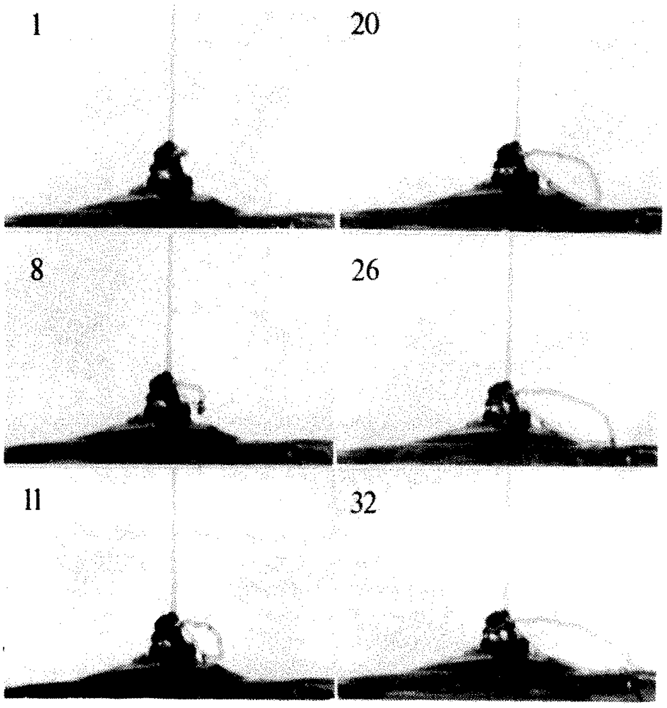
The Kaye effect revisited

WHEN a thin stream of certain solutions of polyisobutylene in dekalin is poured on to an open evaporating dish, a heap of the liquid forms at the point of contact with the dish. As pouring proceeds a small streamer of liquid emerges from the heap, leaving it at a low trajectory, which gradually steepens before the streamer collapses. The whole effect lasts for about one second[1-3]. Using a new filming technique and a film speed of 250 frames s^{-1}, we have observed that the emergent streamer is really a loop of liquid, and we here propose a mechanism for this effect. The second frame in Fig. 1 shows the effect. Such looping of the emergent streamer is not unexpected as the liquid is pituitous. In the following frames the loop extends outwards until the bottom of it strikes the surface of the liquid on the flat glass plate. As the streamer is cutting off the liquid from the heap, the heap collapses and flattens, presenting a smaller angle of incidence to the incident stream and causing the bouncing streamer to steepen in trajectory and move azimuthally, as suggested by Kaye. The base of the streamer's trajectory is often as large as 100 mm.

The decay of the streamer may be caused by the elasticity of the liquid being insufficient to maintain such a steep

Fig. 1 The growth of a loop during the build up of a streamer in the Kaye effect. The frames are 1, 8, 11, 20, 26 and 32 from a 250 frames s^{-1} cine film.



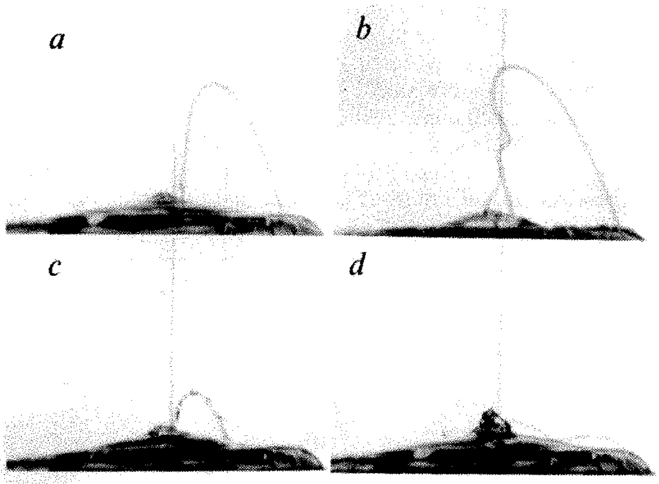


Fig. 2 *a*, The ejection of a kink causing the decay of the Kaye effect; *b*, a collision between the incident stream and the ejected streamer; *c*, coils in the streamer giving a structural weakness and rapid decay; *d*, a secondary streamer caused when the primary streamer strikes the flat glass plate.

trajectory, or by a point of weakness in the streamer, such as a kink that has been ejected from the main body of the liquid (Fig. 2*a*) or, as shown in Fig. 2*b*, by a collision between the streamer and the incoming stream. Once the streamer decays, the heap reforms and the action is repeated.

Some streamers never attain large trajectories and decay after a very short time (~0.5 s). This is often because of coiling within the streamer, making it structurally weaker (Fig. 2*c*). It has not been possible to ascertain whether each coil propagates along the streamer, whether each is stationary with respect to the streamer, or whether the motion of the coils is a combination of both.

Figure 2*d* shows a secondary streamer caused by the emergent streamer striking the thin film of liquid lying on the flat glass plate. Such secondary streamers are quite common.

We suggest the following mechanism for the Kaye effect in a polyisobutylene-in-dekalin solution. The liquid in the heap is moving slowly and its viscosity is high, so that it presents a rigid surface to the incoming stream, particularly as it is backed by the flat glass plate. The liquid in the incoming stream is unsheared during its descent and its viscosity too will be high, but on striking the heap there will be rapid changes in the velocity of the liquid on the surface of the heap giving rise to high shear rates. If the liquid were highly shear thinning as well as elastic and pituitous, its apparent viscosity at high shear rate would be low and its elasticity high, the optimum conditions for the reflection of a streamer from the heap.

This proposal requires that the above liquid be highly shear thinning, and it may account for the critical dropping height necessary to provide the best conditions for the Kaye effect. Measurements were carried out on the variation of the apparent viscosity of the liquid with shear rate in the range $40\ \text{s}^{-1} < \gamma < 120\ \text{s}^{-1}$ using a concentric cylinder viscometer, the most suitable viscometer available at the time. The results show that the solution is a power law fluid of index 0.4, which, although the means of measurement are not ideal, indicates that the solution is highly shear thinning. It is hoped to repeat this measurement over a larger range of shear rate using a cone and plate viscometer at a later date.

On a macroscopic scale the proposed mechanism for the Kaye effect requires that any polymer solution likely to show it should be elastic, pituitous and highly shear thinning. Moreover, the polymer molecules must have flexible backbones, but any hydrogen bonding would be disadvantageous because of the additional interactions between the chains involved.

Our present experiments were conducted on a solution of 4.4 g of polyisobutylene in 100 ml of dekalin, the polymer grade being Visnatex L140 of average molecular weight 2×10^6 (Esso). The solution was dropped from a height of 400 mm on to a flat glass plate.

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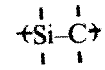
Development of a silicon carbide fibre with high tensile strength

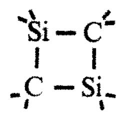
MUCH work has been done on preparing heat-resistant silicon carbide materials in fibrous form, since plastics or metals can be reinforced with them to obtain very heat-resistant material of great mechanical strength. SiC whiskers[1] are, however, impractical because of their shortness (several mm), their non-uniform diameter and high cost of production. SiC-on-W (ref. 2) and SiC-on-C (ref. 3) filaments have been produced by chemical vapour methods. These coated filaments are more expensive, and the treatment for making such composite materials requires careful control. We report here on the synthesis of continuous β-SiC fibres by a new process: the conversion of organometallic polymers to inorganic substances. We have studied the transformation process and the structure and mechanical properties of these fibres.

The synthesis proceeded as follows[4,5]. Dimethyldichlorosilane $(CH_3)_2SiCl_2$, as the starting material, was converted to polydimethylsilane[6], $[(CH_3)_2Si]_n$, by dechlorination with metallic sodium, and was in turn converted to a polycarbosilane polymer,



$$\left(\overset{|}{\underset{|}{\text{Si}}}-\overset{|}{\underset{|}{\text{C}}} \right)_n ,$$

of mean molecular weight, MW, ~ 1,200 by pyrolysis at 400 °C in an inert gas atmosphere. The distribution of molecular weights of the polycarbosilane was measured by solvent fractionation. For a number of systems, it was found that the peak of the distribution curve corresponded almost exactly to the MW of the unfractionated polymer. Three calibrated distribution curves are shown in Fig. 1. The peaks of curves *a*, *b* and *c* correspond to MW=1,086, 1,226 and 1,199, respectively. In our case, it was important for the molecular weight not to be too large. A chemist trying to synthesise a fibre generally desires to produce a polymer chain with a molecular weight from several tens of thousand to ~ 100,000. The ring opening polymerisation of disilacyclobutane



does give a giant molecule of molecular weight 10,000–100,000 (refs 7–9) but it cannot be dry- or melt-spun because of its liquid or gummous state. On the other hand, the resinous polycarbosilane polymer obtained from pyrolysis of polydimethylsilanes can be

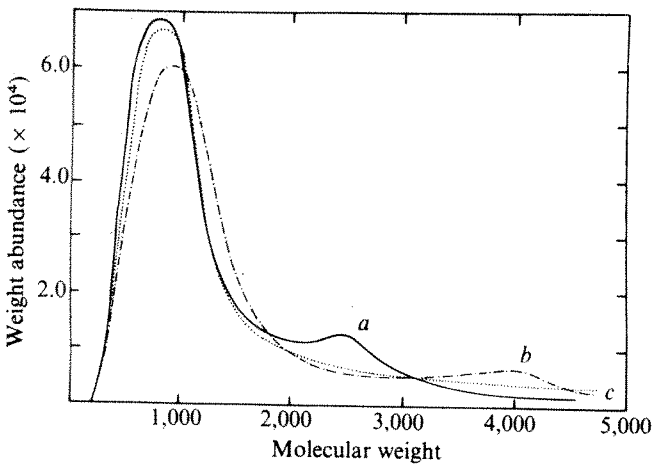


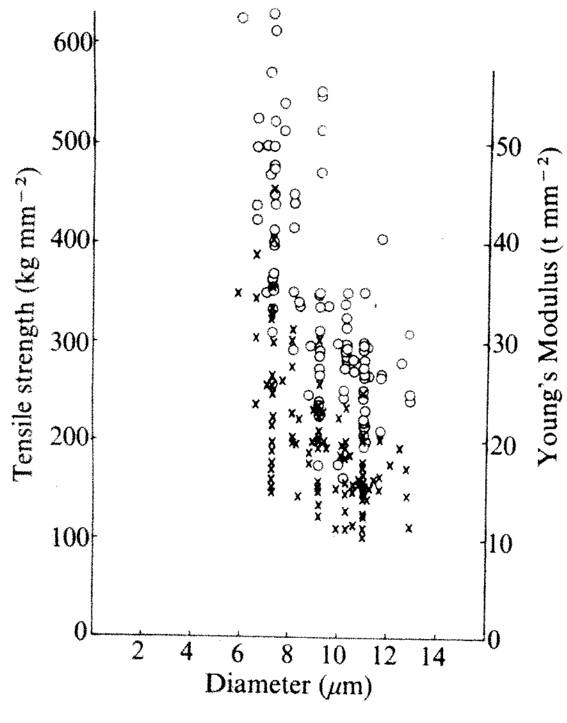
Fig. 1 Calibrated molecular weight distribution curves of polycarbosilanes

melt-spun, and the tensile strength of the starting fibre (8–20 μm in diameter) is very low $(0.5–1)\times10^7$ N m^{-2}. Heating a bundle of the fibres to ~ 200 °C in air produced a thin silicate film over surface of the fibres; this was done to protect the fibres from melting later on. To break up organic bonds, such as Si–CH_3 and C–H, the polycarbosilane fibre was then heated at up to 1,500 °C in vacuum and thereby converted into a continuous SiC fibre with a tensile strength of 62×10^8 N m^{-2} and a Young's modulus of 4.4×10^{11} N m^{-2} for a diameter of ~ 7 μm.

Infrared spectroscopy and thermogravimetry (TG) of the polycarbosilane from room temperature to 1,300 °C indicated that the decomposition occurs gradually above 300 °C and is completed at ~ 800 °C. Since ~ 60% of the material by weight is left, this suggests that organometallic and organic bonds, for example, Si–CH_3 and C–H, decompose, so that the polycarbosilane is converted into a material containing mainly Si–C bonds[4,5]. A little carbon is produced as a by-product, but it is given off with partial oxidation.

The length and diameter of the SiC fibre can easily be controlled by the spinning process. The relation between the diameter of the SiC fibre and tensile strength and Young's modulus is shown in Fig. 2. The values obtained by this method are both higher than those previously reported[4,5], and are seen to approach those of β-type silicon carbide whisker. We consider this to be caused by the difference of the preparation in the spun polycarbosilane.

Fig. 2 Tensile strength (○) and Young's modulus (×) of β-SiC.



The gradual changes in the X-ray diffraction patterns were measured from room temperature up to the highest temperatures to examine the structural changes going from polycarbosilane to SiC fibre. The broad patterns obtained with CuKα (Ni filter) are shown in Fig. 3. As the polycarbosilane fibre is heated, the first peak decreases and completely disappears at above 1,000 °C. The second and third peaks in Fig. 3 decrease for the sample heated at ~ 800 °C, but are nevertheless observed for all samples. As the polycarbosilane fibre is heated to higher temperature, those peaks are shifted to a slightly higher angle, the 111 pattern changes, and the 220 and 311 patterns of β-SiC are seen. The polycarbosilane ceases to decompose at ~ 800 °C and is gradually changed into β-SiC when heated to above 800 °C. Infrared, TG and

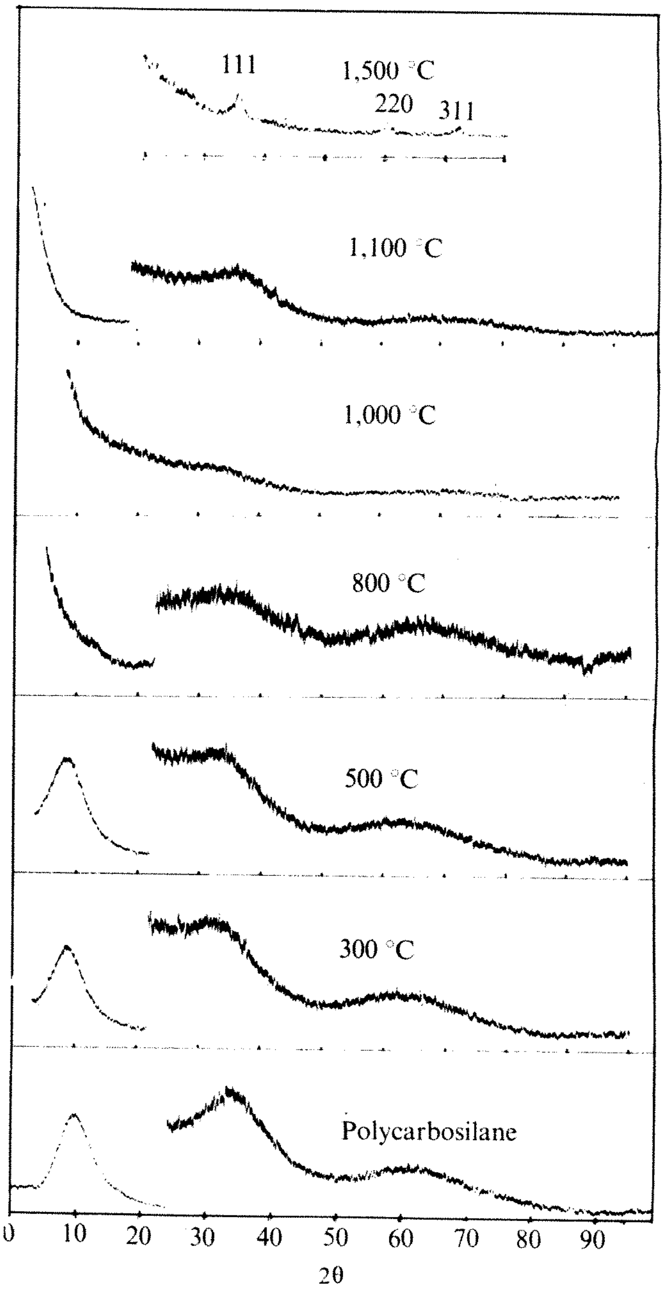


Fig. 3 X-ray diffraction patterns of polycarbosilane and β-SiC fibres heat treated at several temperatures.

X-ray analysis showed the Si–C skeleton in the polycarbosilane to be converted to the β-SiC structure. From observations with an ultrahigh voltage electron microscope and the X-ray diffraction patterns, we found the SiC fibre heated at 1,500 °C was polycrystalline, made of fine particles of diameter ~ 70 Å. X-ray transmission pinhole photographs of the fibre showed no fibrous texture.

On the other hand, the 30-μm SiC fibre heated at 1,200 °C was held under a load of 50 g for 3 d in air and showed no sign of breaking. It may well have revolutionary applications as a reinforcing fibre viable up to at least 1,200 °C.

The fine particles of diameter ~ 70 Å cohere tightly to give the fibre. That is, several tens of the polymer molecules (MW 1,200), as described above, assemble to form the inorganic β-type SiC. It is not clear why a high tensile strength should be associated with a SiC fibre composed of fine crystallites, for the tensile strength must be a function of large cohesive forces in the assemblage of fine particles. How can the cohesive force in the assemblage of fine particles be large?

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Low level determinations of environmental cadmium

THE discovery that environmental levels of mercury and lead present serious health hazards has led to intensified investigations into the effects of other heavy metals. Cadmium is a particularly toxic contaminant, and as with mercury, widespread environmental exposure to cadmium is related to its increased technological use[1]. We have developed an isotope dilution technique to measure the concentration of cadmium in aqueous solutions with a sensitivity of 0.003 μg l^{-1}. The concentration of cadmium in two river systems in Western Australia has been measured to establish an accurate set of low level reference determinations in a region which is relatively free of sources of industrial pollution.

Williams and David[2] have shown that the cadmium content of phosphatic fertilisers and superphosphate used on Australian pastures range from 27 to 90 p.p.m., and that the application of superphosphate is reflected in an increasing cadmium content of the soil. Much of this cadmium is soluble in water and may therefore be transferred to natural waterways. Waterways are of concern because reservoirs may be sited on them and be a source of drinking water and irrigation supplies. The World Health Organization (WHO) has declared that the maximum advisable concentration limit for cadmium in drinking waters is 10 μg l^{-1} (ref 3), whereas the recommended maximum level for irrigation purposes is 5 μg l^{-1} for continuous use[4].

Water samples were collected in high density polyethylene containers since it has been shown[5] that polyethylene does not adsorb cadmium from aqueous solutions and exhibits a low cadmium blank. A 200-g aliquot of each water sample was spiked with an accurately known weight of isotopically enriched ^{111}Cd tracer. After ensuring that the spike and sample were well mixed, the cadmium was chemically extracted by ion exchange. The extracted samples were analysed in a solid source mass spectrometer, and from the measured isotopic ratios the concentration of the cadmium was determined. Details of the stable isotope dilution technique used in this project are described elsewhere[6].

Two river systems were selected for study The Swan River and its tributaries (the Avon, the Helena and the Canning Rivers) form the major river system for Perth and the surrounding metropolitan area. Two major reservoirs—the Canning and Mundaring Weir—are situated on tributaries of the Swan River. The mouth of the Swan River is at Fremantle and for ~ 35 km the river is tidal and salty. The second river system studied in this project was the Peel Inlet System. The Peel Inlet is situated some 50 km south of Perth and is a large body of salt water connected to the sea by a narrow channel. The Murray River and its tributaries flow into the Peel Inlet. The surrounding area is mainly rural and embraces an extensive irrigation district, although in the close vicinity of the Peel Inlet there is a residential area.

Western Australia is a very isolated region, and very little industrial development has taken place in the area covered by this study. The narrow coastal plain is terminated by the Darling Scarp which lies parallel to the coast. This 300-m high escarpment is the western margin of the Yilgarn Block, which is the largest area of Archaean rocks in Australia[7]. The Yilgarn block comprises remnants of metamorphosed volcanic and sedimentary rocks intruded by granitic rocks. The concentration of cadmium in the rock types comprising the Yilgarn Block is ~ 100 ng per g (ref. 8) Most of the region drained by the two river systems is natural forests and farmlands, except for the residential areas around Perth and Mandurah.

The river systems are shown in Fig. 1. The numbers refer to sample locations on the two river systems, details of which are given in Table 1. Altogether 38 water samples were collected from the Swan River System and 14 from the Peel Inlet System. Each sample was analysed in duplicate. The measurements for each region in the river systems have been averaged to give a mean cadmium concentration for each region. The sensitivity of the technique used in this study is 0.003 μg l^{-1}, and in most cases the accuracy of a single determination is ± 10%.

The cadmium concentration near the mouth of the Swan River is 0.10 μg l^{-1} and is in good agreement with the concentration in normal seawater (0.11 μg l^{-1} (ref. 9)). Near Perth the concentration increases to 0.30 μg l^{-1} where the

Table 1 Cadmium content of river systems

Sample no. (see Fig 1)	No. of samples analysed	Locality*	Mean concentration (μg l^{-1})
1	5	Mouth of Swan River	0.10
2	3	Upstream from Perth	0.30
3	9	Middle Swan District	0.09
4	7	Avon River	0.05
5	7	Helena River	0.02
6	7	Canning River	0.05
7	7	Peel Inlet	0.04
8	7	Murray River System	0.05

*The samples were collected in May 1975. There was little rainfall in the 6 months preceding sample collection.

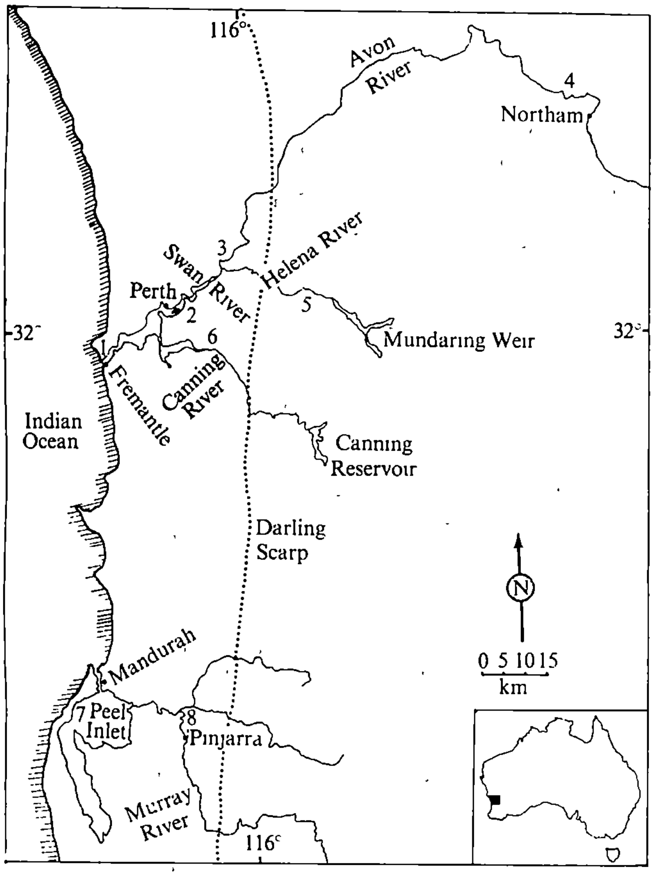

Fig. 1 Sample locations for the Swan and Peel Inlet River Systems in Western Australia.

samples were collected in the vicinity of an industrial area (the maximum value being 0.44 μg l^{-1}).

The Middle Swan District is situated among vineyards in a semi-rural area and here the cadmium content is <0.1 μg l^{-1}. Further upstream the concentration decreases still further, and in the farming area around Northam it is only 0.05 μg l^{-1}, indicating that the cadmium concentration of this natural waterway is little affected by the presence of fertilisers in the surrounding soil. The Helena River samples were collected near the boundary of a forested catchment area and gave the lowest average value of all the samples of 0.02 μg l^{-1}. The Canning River is used extensively for recreational purposes, and the surrounding area is residential and semi-rural. The average value is 0.05 μg l^{-1}, but the individual concentrations are lower upstream.

The Peel Inlet exhibits a low concentration, but this probably arises from the fact that some of the cadmium has been adsorbed on the underlying sediments[10], and reflects the fact that little exchange of water has taken place with the sea. The Murray River system exhibits a slightly higher value of 0.05 μg l^{-1}, which is consistent with the cadmium concentration in the Avon River in the other farming area near Northam.

The results of this study demonstrate that the cadmium content in the two major river systems in Western Australia is ~100 times lower than the WHO limits. The data indicate that the cadmium content tends to decrease upstream from the mouth of the river, and that in the reservoir catchment areas the cadmium content is as low as 0.02 μg l^{-1}. These values are substantially lower than waterways in other parts of the world[11-13], where the cadmium concentration is often > 1 μg l^{-1} We hope our results will provide a benchmark for the comparison of future measurements on the World's waterways.

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Correlations between concentrations of elements in plants

INTEREST in the cycling of the elements through ecosystems has increased in recent years, and numerous plant species have been analysed. It can be assumed that elements do not cycle independently in vegetation because they are involved in the basic structure and function of cells, which have similar biochemical pathways. Thus concentrations of different elements must be correlated. Little attention has been given to the relationships between concentrations of elements within or across a variety of species. This is undoubtedly because the chemical composition of individuals within a species may vary considerably from one site to another[1]. The ratio of different elements may be relatively unaffected by this geographic variation because natural vegetation can absorb and accumulate nutrients selectively[2] maintaining a balance of intracellular concentrations to optimise metabolism, protein synthesis and tissue production. If concentrations of elements were correlated across a variety of species from different sites, this would indicate a relatively constant ratio between concentrations of elements among plant cells. I report here correlations between concentrations of nitrogen, phosphorus, magnesium, calcium and potassium, across a variety of species, which seem to be based on biochemical similarities between elements in cell metabolism.

Mean concentrations of P, N, K, Ca and Mg in non-vascular plants, vascular plants and aquatic macrophytes were examined for correlations between 54 species. Data were obtained from the literature and unpublished sources, and few restrictions were placed on the data set. Differences in the analytical techniques used were ignored. Sampling times in the studies examined had ranged from April to November but, mostly, analyses had been made on plants collected in summer or autumn. Concentrations were calculated as percentage dry weight.

Non-vascular plants included species of 14 algal genera collected from April to November in Alabama and Mississippi: *Anabaena*, *Aphanizomenon*, *Chara*, *Cladophora*, *Euglena*, *Hydrodictyon*, *Lyngbya*, *Microcystis*, *Mougeotia*, *Nitella*, *Oedogonium*, *Pithophora*, *Rhizoclonium* and *Spirogyra*[3]. Two moss species collected in midsummer were also included: *Sphagnum magellanicum* from near Ottawa, Canada[4] and *Philonotis fontana* from New Hampshire[5]. Eleven species of aquatic macrophytes were included: data from Boyd[6] on eight genera (*Elodea*, *Myriophyllum*, *Vallisneria*, *Potamogeton*, *Lemna*, *Paspalum*, *Najas* and *Phragmites*) and on three genera from the south-eastern Unites States (*Eichhornia*[1], *Justicia*[7,8] and *Pistia*[9]).

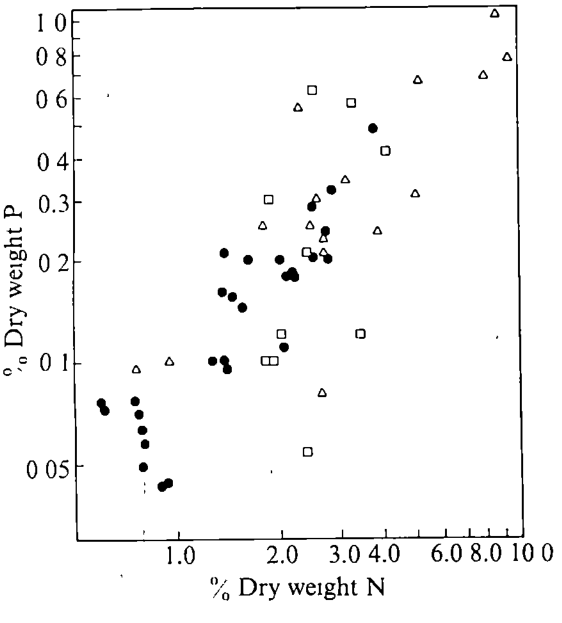
Concentrations of elements were obtained from leaves of vascular species. There were eight woody species from Brookhaven, New York (*Quercus coccinea*, *Q. alba*, *Q. ilicifolia*, *Pinus rigida*, *Gaylussacia baccata*, *Vaccinium vacillans*, *V. angustifolium* and *Kalmia angustifolia*)[10], two woody species from central Oklahoma (*Quercus stellata* and *Q. marilandica*)[11] and six species from New Hampshire (*Acer saccharum*, *A. spicatum*, *Betula alleghaniensis*, *Fagus grandifolia*, *Picea rubens*, *Prunus pensylvanica*)[5]. Data on four herbaceous species from the south-eastern United States were also included: *Saururus cernuus*[12], *Juncus effusus*[13], *Typha latifolia*[14] and *Alternanthera philoxeroides*[7,8]. Finally, there were unpublished data for woody and herbaceous species from South Carolina: *Alnus serrulata*, *Myrica cerifera*, *Salix nigra*, *Scirpus cyperinus*, *Erianthus* sp., *Polygonum punctatum* and *Sagittaria latifolia*.

Phosphorus and nitrogen concentrations were correlated significantly across the species examined ($P<0.001$, Fig. 1). Within the three broad categories of plants, correlation between P and N in vascular and non-vascular plants was significant ($r=0.90$ and 0.85 respectively, $P<0.001$) P and N were not correlated significantly in the aquatic macrophytes ($r=0.46$, $P>0.05$). When P concentrations were regressed against N concentrations, the slopes of the regression lines were similar for all three categories. The regression coefficients ($b\pm1$ s.d.) were as follows. non-vascular plants, 0.095 ± 0.016; aquatic macrophytes, 0.105 ± 0.071, and vascular plants, 0.108 ± 0.010. The intercept value in each of these regressions and in the overall regression across species was zero.

Ca and Mg concentrations were correlated significantly in non-vascular plants ($r=0.65$, $P<0.01$), aquatic macrophytes ($r=0.78$, $P<0.01$) and vascular plants ($r=0.48$, $P<0.05$) and across all species ($P<0.001$, Fig. 2). When Mg concentrations were regressed against those of Ca, the slope values ($b\pm1$ s.d.) were 0.089 ± 0.029, 0.124 ± 0.033 and 0.143 ± 0.051 for non-vascular plants, aquatic macrophytes and vascular plants, respectively. A correlation matrix among N, P, K, Ca and Mg concentrations across all species was calculated. Correlations between other element pairs were not statistically significant ($P>0.05$), except for a correlation between Mg and K ($r=0.50$, $P<0.001$).

Correlations between pairs of elements have been reported before in laboratory studies but not for natural vegetation. In ryegrass, *Lolium perenne*, grown in nutrient solutions containing various concentrations of sulphate and nitrate, a relationship was observed between gram atoms of protein S and protein N (ref. 15). Both S and N are important constituents of proteins and amino acids. Similarly, N and P are closely associated in both cytoplasmic and nuclear material, including cell membranes, nucleic acids and ATP, ADP and NADP. Correlations between N and P reflect their close association in plant biochemistry, particularly protein synthesis. The lack of correlation between N and P in aquatic macrophytes may be due to the narrow range of N values reported here, or to other factors such as "luxury consumption"[16] of N when environmental concentrations of P are deficient. A relatively constant ratio of P to N (1:10) across species and types of plant suggests a very general association between these elements.

Fig. 1 Relationship between concentrations (% dry weight) of P and N across 54 species Only leaf concentrations were used from vascular plants Correlation is significant at the $P < 0.001$ level. $r = 0.84$, $b\pm1$ s d. $= 0.10\pm0.009$ ●, Vascular; □, aquatic macrophytes, △, non-vascular.



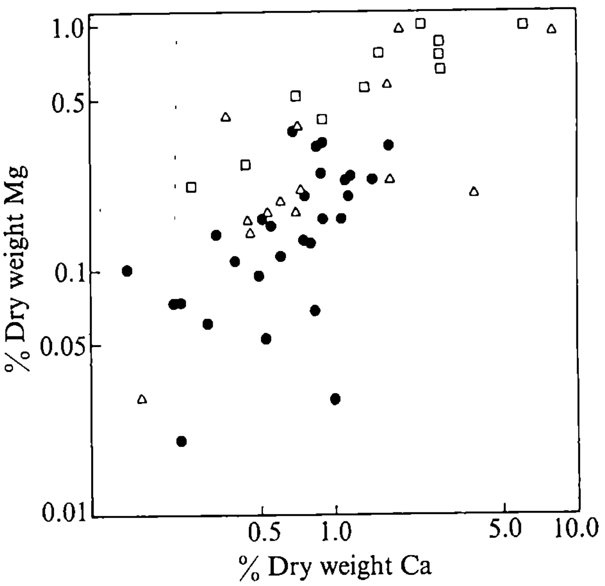


Fig. 2 Relationship between concentrations (% dry weight) of Ca and Mg across 53 species. Only leaf concentrations were used for vascular plants. Correlation is significant at the $P < 0.001$ level. $r = 0.70$, $b\pm1$ s.d. $= 0.13\pm0.019$. Symbols as for Fig. 1.

Calcium and magnesium have structural and enzymatic functions in the plant cell. Mg is central to the structure of chlorophyll, and Ca is a component of pectins in the cell wall. The correlations between Ca and Mg concentrations is probably a consequence of the close association between these elements in metabolism and photosynthesis[16]: both elements are enzyme activators in metabolic reactions. As chloroplasts may contain 50% or more of the total leaf Mg and 60% of the total leaf Ca[17], a correlation between the concentrations of Mg and Ca across species is to be expected. The ratio of Mg:Ca appears to be more variable than that of P:N across different plant types. Over all species, the Mg:Ca ratio was 1:7.7. Mg and K, which were also correlated, are essential for certain enzyme activities in all plants and both are linked to photosynthesis[16]. Furthermore, concentrations of these elements could be correlated because of similar chemical properties. N and P share common valence states, as do Ca and Mg. Their mechanisms of uptake across cell membranes are probably similar.

There have been previous attempts to find P:N and Mg:Ca ratios that maximise crop yields in agricultural plants[18] but little work has been done with natural species. Gerloff and Krombholz[19] found that the critical contents of N and P—the minimum contents in plant tissue associated with maximum growth—for several species of aquatic angiosperms were 1.3% and 0.13% respectively. For spruce and pine seedlings, the critical concentration of N is approximately 1%, and that for P is 0.08–0.1% (refs 20 and 21). The critical P:N ratio seems to be 0.1, which is the same ratio I have found using many more species. The evolution of constant ratios of elements may have been related to the maintenance of biochemical equilibria within cells that maximise protein synthesis and tissue production in natural environments.

Undoubtedly, concentrations of elements in the environment, as well as a combination of edaphic or biological factors which result in luxury consumption or nutrient deficiencies of elements in leaves, can produce a correlation or lack of correlation of concentrations in plants. But in view of the number of species and variety of sites included in this analysis, similarity in the biochemical function of pairs or groups of elements seems to be the most important factor contributing to the observed correlations.

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Adjusting to the changes to and from Daylight Saving Time

A GREAT deal of attention has recently been paid to the circadian rhythms that have been found to exist in a number of human physiological and behavioural processes. There are several ways in which such rhythms can be disrupted, and extensive studies have been made of the effects of prolonged isolation[1], abnormal working hours[2], and flight across several time zones[3]. The adoption of Daylight Saving Time (DST) is another possible cause of disruption, but seems to have been generally ignored in spite of the large number of people potentially affected. We have found that significant disruptions of behaviour occur during adaptation to the time change.

More than 25 countries around the world now use a DST system of one kind or another. The system used in Britain comprises a notional shift in time zone, eastward (that is "losing" 1 h) in the spring and westward (that is "gaining" 1 h) in the autumn. Although the general effect of DST on energy consumption and behaviour patterns has been studied quite extensively[4], we can find no studies reporting the disruptions in behaviour that might be produced by the actual time changes themselves.

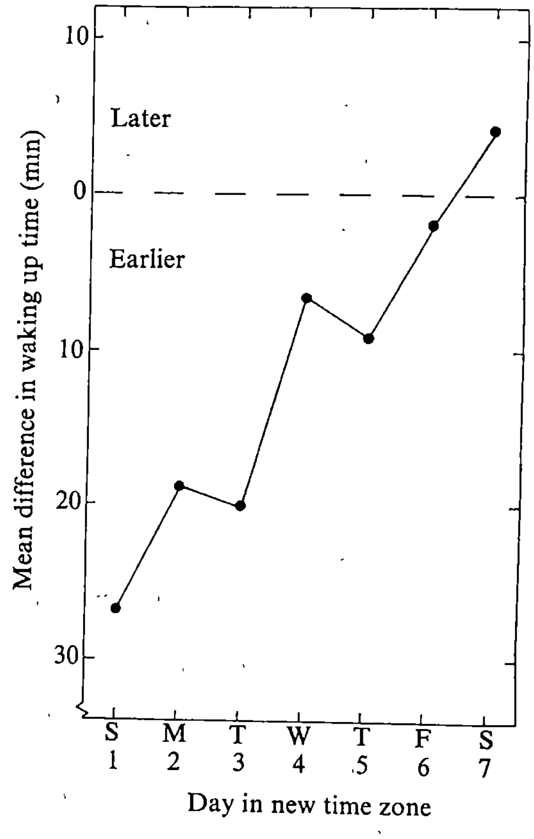
The high cost of "jet lag" studies usually requires that fairly large shifts in time zone are considered, since comparatively few subjects can be used, and there tend to be large individual differences. Time zone shifts of less than 3 h have thus typically been ignored. In the changes to and from DST, the investigator is presented with a whole population that is trying to adapt to a "new" time zone. We therefore considered the changes to and from DST to be ideal opportunities for the study of adaptation to small shifts in time zone using a relatively large number of subjects.

The study was conducted during the change back from British Summer Time (BST) to Greenwich Mean Time (GMT) in the autumn of 1974. At 0200 GMT on Sunday October 27, the clocks were officially moved back 1 h (that is from 0300 BST to 0200 GMT). Sixty-five subjects (55 of them female) living in the Brighton area took part in the study. For 6 d before the DST change and 11 d after it, the subjects were asked to record their time of waking; and at 0900 (clock time), to measure their oral temperature (by a 3-min sublingual insertion of a standard clinical thermometer) and rate their feelings of alertness on a scale from 0 to 20.

The process of adaptation to the time change was most apparent in the mean waking up times. Since an hour was effectively "gained" by the change, earlier waking up times than normal were expected. When the waking up times of the days following the DST change were compared with those of a normal week a clear disruptive effect was observed (Fig. 1). This effect was still statistically significant on the fifth day in the "new time zone" ($t = 1.75$, d.f. = 38, $P < 0.05$). The subjective alertness scores at 0900 were, as expected, positively correlated with the length of time awake ($r = +0.8$, d.f. = 15, $P < 0.01$), and there was a corresponding slight increase in the ratings of perceived alertness during the week following the DST change.

The circadian rhythm in oral temperature normally produces a rise of about 0.1 °C from 0900 to 1000 (ref. 5). Since, after

Fig. 1 The mean difference in waking up time between a day of the week following the DST change and the corresponding day of a week unaffected by the change. Each point represents the mean difference of the 39 subjects who returned waking up times for all 17 days of the study (a similar curve was produced when all 65 subjects were included.)



the time change, the readings were actually taken at 1000 as defined by the "old" time zone, any lack of adaptation in the rhythm would be indicated by a corresponding rise in temperature. Unfortunately there were marked fluctuations in the air temperature at Brighton over the 17 d and the mean oral temperature of the 65 subjects was found to be significantly correlated with these fluctuations ($r = +0.7$, d.f. = 15, $P < 0.01$). The presence of such correlations and other uncontrolled factors, such as the menstrual cycle of the female subjects, makes interpretation of the oral temperature data rather difficult. Nevertheless, when the effect of air temperature was partialled out using a linear approximation, a rise in adjusted oral temperature was found in the 6 d following the DST change.

The process of adaptation in waking up times took much longer than the one day (or less) that would be predicted from studies of the "jet lag" effect[6]. A possible reason for the discrepancy is that in travel across time zones both the physical cues such as sunrise and sunset and the social cues such as working, eating and sleeping habits, change in a way that encourages adaptation to the new cycle. In the DST change, however, only the social cues change, the physical ones remaining synchronised to the "old" cycle. While much of the recent work has emphasised the importance of social cues over physical ones, it is possible that the latter may have impeded the process of adaptation.

This study was concerned with a westward (autumn) shift in time zone. Studies[3] of the jet-lag effect have suggested that eastward shifts are the more disruptive and an even greater disruption might thus be expected in the DST change that occurs in the spring. Since "jet lag" has been shown to impair performance[3], it is possible that the disruption induced by DST changes (particularly in the spring) may have a similar effect. A preliminary study was thus undertaken to determine whether this disruption was sufficient to be associated with a measurable increase in road accidents. Evidence of an increase during the week following the spring DST change was found, but further validation of this result is clearly required.

In conclusion, it seems that adjustment to the time changes associated with DST is not instantaneous, and that significant disruptions in behaviour may occur during adaptation to the new cycle. The presence of such disruptive effects following a 1-h transition reinforces the importance of studying shifts in time zone, even when the time change involved is considerably less than that typically encountered in transcontinental flight.

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Visibility of flicker in television pictures

A PRIME requirement in a television system is that the flicker inherently associated with the displayed picture should be minimal. The visibility of such flicker is generally considered to depend primarily on the picture luminance, the angle subtended by the picture and the field repetition frequency. The field frequency should therefore be chosen to be close to, and ideally higher than, the fusion frequency for the human visual system. With the display luminances generally encountered in normal broadcast television, field frequency flicker is negligible in countries using 60 fields s^{-1}, and, though often noticeable, has proved acceptable in those using 50 fields s^{-1}.

It was somewhat surprising, therefore, while investigating the effect of line and field scanning directions on the legibility of alphanumeric television displays with luminances which were higher than normal, to find that the flicker was more visible when the field was scanned from left to right (L–R), right to left (R–L) or bottom to top (B–T) than it was for the normal top to bottom (T–B) direction. A closer scrutiny seemed to indicate that the increased visibility was due to greater sensitivity to the movement of the luminous emittance through the display in these directions of field scan. No previous report of this phenomenon is known to the authors, and the following subjective experiment was carried out to verify its existence in quantitative terms.

A vidicon camera channel, operating at 625 lines per picture and 50 fields s^{-1}, and used with a slide projector, provided a source of still pictures. A facility for reversing the direction of field scan was a standard feature. A 350-mm television picture monitor was modified to allow the direction of field scan to be switched in synchrony with the picture source so that the displayed picture always appeared the right way up. A 500-mm square matt-grey mask with a 210-mm square cut-out was positioned in front of the monitor. The 1 : 1 aspect ratio allowed field scans (L–R) and (R–L) to be produced, with the displayed picture dimensions unchanged, by turning both the slide and the monitor (behind the mask) through 90°.

The average illuminance of the specially equipped viewing room, measured at table height, was 50 lx. Gathered white curtains, subtending a horizontal angle of about 120° at the observer's eyes, were used as a backcloth, and concealed lighting was adjusted to give a backcloth luminance of 3.5 cd m^{-2} over a large area behind the monitor. The luminance of the grey mask surround was 1.6 cd m^{-2}.

Four pictures were chosen from a large available stock to exhibit from well above average to about average degrees of flicker. They comprised two head-and-shoulders portraits, one scene and one black line drawing on a white background. The highlight luminance of the display monitor was set to 200 cd m^{-2}, which, in conjunction with the luminance of the inactive screen, 1 cd m^{-2}, defined the maximum available contrast ratio of 200 : 1.

The observers were colleagues working in the fields of television and photography. Pairs of observers attended each test session, and were seated at a table in front of the monitor at a viewing distance of 1.26 m, six times the picture height. The centre of the screen was at a height of 1.05 m and was therefore a little below eye level for the average seated observer.

Initially, two tests were planned—one in which the (T–B) and (B–T) directions of field scan would be present, and the other for the (L–R) and (R–L) directions. To the extent that interlace might affect the visibility of flicker, the presence or absence of interlace was included as an additional variable, the non-interlace scanning standard being 312 lines per picture, 50 pictures s^{-1} A balanced test design ensured that each picture was associated with each combination of scan direction and interlace condition, and replication gave a total of 32 presentations per viewing session. Where possible, the same 24 observers attended each test, and each of the 12 viewing sessions in each test lasted on average 20 min. The observers were asked to assess the flicker using the following 5-grade scale: imperceptible; perceptible, but not annoying; slightly annoying; annoying; very annoying.

To eliminate the remote possibility that the variation of visibility with field scan direction was an equipment effect and not a visual perception effect, a third test was carried out with (T–B) and (B–T) scan directions as in test 1, but with both the display monitor and the slides turned upside down. Any contribution to the flicker caused by the equipment would thus move through the pictures in opposite directions in tests 1 and 3. Also, a check made by a small number of observers with the room lights turned off confirmed that no significant flicker effects were being caused by the interaction of the display field frequency and the room illuminance frequency.

Results are quoted in terms of the mean opinion score, obtained by allocating the values 1, 0.75, 0.5, 0.25 and 0 to the grades "imperceptible" through to "very annoying" and calculating the mean value for each picture condition. An analysis of the sources of variance in the results for the individual pictures in all three tests indicated that the interlace and the replication factors did not contribute significantly to the total variance. Thus, in all three tests, average values of mean score were obtained for each picture/scan direction combination. These averaged values in tests 1 and 3 showed no significant or systematic differences and could be further averaged, indicating that the phenomenon being investigated was truly of a visual perception nature. For similar reasons, the results for (L–R) and (R–L) directions of scan in test 2 were averaged. The average mean scores are plotted as functions of the average picture luminance in Fig. 1. The kink in each curve indicates that average luminance is not the most appropriate objective measure of susceptibility to flicker, but it is easy to define and measure. Furthermore, the main interest is in the relative vertical displacements of the curves and the horizontal scale is mainly a convenience to separate the effects on the different pictures. The confidence interval associated with each data point represents plus and minus one standard error in the mean value, and the differences between the data points for the normal (T–B) scan direction and the corresponding data points for the other scan directions are all highly significant ($P<0.001$) except for one case, easily identified in Fig. 1, where the difference is not significant.

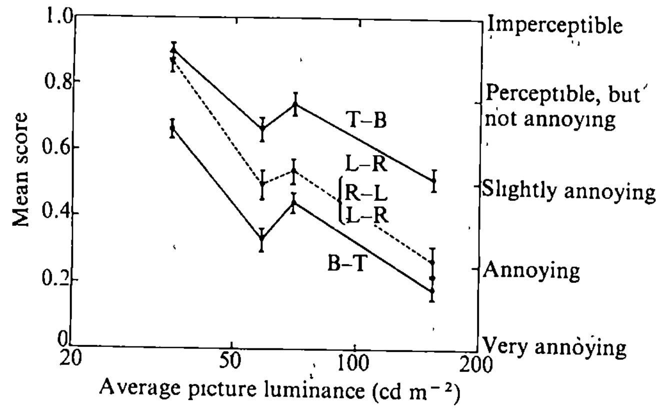

Fig. 1 Visibility of flicker for the various directions of field scan. ——, Average of tests 1 and 3; – – –, test 2.

A fuller investigation and explanation of this phenomenon must be left to those working full time in the field of human visual perception. One possible explanation is that, because of television viewing experience, people have adapted to 50-Hz flicker with the field scanned from top to bottom. Such adaptation would have to be a more powerful and long term effect than that usually associated with continuous movement in one direction; after a few minutes exposure to upwards movement, the associated flicker was still more visible than that apparent the instant the movement was switched to its normal downwards direction.

The existence of such a long term adaptation effect might be an additional reason why visitors from countries with 60 fields s^{-1} television, who have not adapted to 50-Hz flicker, apparently find it so much more annoying. Indeed, one of the reasons for describing the experimental arrangements in some detail is the hope that the experiment may be replicated, still using 50 fields s^{-1} television, in a country where the normal television standard is 60 fields s^{-1} or, preferably, where no television service exists. The results of such an experiment might indicate whether the phenomenon is acquired, which seems more likely, or is inherent. A qualitative check with 60 fields s^{-1} television indicated that the movement associated with the flicker was again more visible in the upwards direction. The magnitude of the increase seemed to be smaller than for 50 fields s^{-1} television, but its existence suggests that any adaptation effect is not precisely linked to velocity.

Meanwhile, confirmation of the existence of the effect suggests that the normal (T–B) direction of field scan should be preferred for new visual telecommunications systems, particularly where a 50 fields s^{-1} standard and high display luminance are envisaged.

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Differing emotional response from right and left hemispheres

To study what might be called the 'emotional vision' of the right and left hemispheres of the human brain, a specially designed contact lens was used to show cine films to the right or left hemisphere only. Films were chosen to provoke different kinds of emotional response. We wished to find whether the two hemispheres would take and utilise the stimulus of the film to generate a different emotional response despite the common input. In recent years clinical and experimental data have supported the hypothesis that different emotional reactions follow damage to the right or the left hemisphere[1–5]. We report here the first part of an investigation in which films were perceived by different hemispheres in normal subjects and the subjects were then asked to judge and rate the films. There is of course no intention of suggesting that information implanted at one hemisphere cannot be transferred to the other[6,7]; nevertheless, the basis of this research is that the two hemispheres can differ in their vision of the world and that each in some respects formulates its own separate and distinct emotional vision of what it sees. We seek to answer the question of what visual experience is like when that which the subject sees enters his nervous system on only one half of his brain, but this is part of the much wider question of how the two hemispheres of the brain see the world and of the relationship between the two visual halves of the brain.

As a preliminary to the experiment reported here a pilot study was conducted in which seven different films were shown to 14 subjects using free vision to assess the nature of their response. Each film was rated on the four dimensions of humorous, pleasant, unpleasant and horrific, using a scale marked 1–9. On the basis of the ratings given, three films were selected for subsequent use. These were a Tom and Jerry cartoon film (duration 3.20 min), a medical film depicting a surgical operation (3.30 min) and a travel film of Lucerne (3.20 min). The sound tracks were not used.

The volunteer subjects were students aged between 18 and 24. All were normally contact lens wearers. They were all right-handed as judged by the Annett handedness questionnaire. Each of the 21 subjects was assigned alternatively to either the left or right visual field. The films were shown to one hemi-

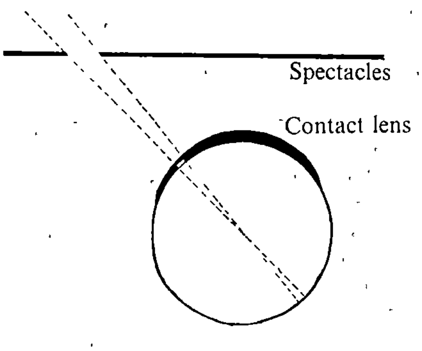

Fig. 1 The optical system.

sphere only for each subject. The order in which the film clips were shown was balanced according to a Latin square design.

The lenses were first inserted and checked for accuracy of fitting. The subject's own spectacles were worn to provide for corrected vision. These were blacked out as we shall describe. Light sensors were fitted to the ear to record heart rate and electrodes to the fingers to record electrodermal response. The results obtained from these measures will be reported on a separate occasion. A check was made that the subject could see the whole area of the screen. The subject was then instructed to relax and watch the film. At the conclusion of each film the subject was asked to rate it on a scale of 1–9 on the four dimensions of humorous, pleasant, horrific or unpleasant. This was done manually on the form provided, the subject continuing to wear the contact lenses meanwhile.

The lenses were constructed of a rigid plastic. They were plano, and of the following dimensions: back optic curve—8 mm: 8.5 mm: 10.5 mm: diameter—7 mm: 7.5 mm: oval diameter—10.0 mm. The lenses were opaque except for a 1-mm wide vertical slit decentred 2 mm from the optic axis. For left hemisphere vision the slit was decentred 2 mm to the right, for right hemisphere vision 2 mm to the left. Both lenses were truncated 1.5 mm. The truncated edge of the lens rested on the lower lid of the eye which prevented the lens from rotating, thus maintaining the vertical slit in a stable position throughout the experiment.

Vision was binocular throughout, the subjects wearing lenses in both eyes. The subjects wore their own spectacles in conjunction with the contact lens during the tests. The corresponding lens of the spectacles was covered with black insulating tape to leave a clear slit corresponding to that of the lens and on the same side. This was done to provide what we at that time regarded as an additional safeguard against stray light entering the eye from an undesirable angle (Fig. 1).

The results are presented in Table 1. This gives the mean judgment for each film on the 1–9 scale. Ratings for films viewed on the right hemisphere are compared with those viewed on the left hemisphere. These in turn are compared with the results of the pilot study in which the films were shown in free vision to 14 subjects and rated according to the same criteria.

Table 1 Response to film viewing on the right hemisphere (RH), the left hemisphere (LH) or in free vision (control)

Humorous	Tom and Jerry	Travel	Medical	Total
RH	6.00	1.80	1.80	9.60
LH	6.27	1.27	1.00	8.54
Control	6.57	1.50	1.00	(9.07)
Unpleasant				
RH	2.80	3.50	6.00	12.30
LH	1.45	1.45	5.45	8.35
Control	1.42	1.07	6.85	(9.34)
Horrific				
RH	2.50	1.50	5.00	9.00
LH	1.54	1.36	3.54	6.44
Control	1.71	1.07	3.85	(6.63)
Pleasant				
RH	6.40	6.20	2.30	14.90
LH	6.72	5.81	2.54	15.07
Control	6.14	6.92	2.07	(15.13)

A Friedman two-way analysis of variance performed on the data shows that there are significant differences between the left hemisphere, right hemisphere and control conditions ($\chi^2 = 32.91$; $P < 0.001$). When comparison was made between the right and left hemisphere significant differences were found for the judgment of unpleasant for all films, the right hemisphere judgment as more unpleasant than the left (Tom and Jerry, $U = 71$, $P < 0.01$ level; Travel, $U = 73$, $P < 0.01$; Medical, $U = 53$, $P < 0.01$, Mann–Whitney U test). Significant differences were also found for the horrific category (Tom and Jerry, $U = 77$, $P < 0.01$; Medical, $U = 51$, $P < 0.01$; Mann–Whitney U test). Significant differences in this category were not found for the travel film.

No significant differences were to be observed for the categories humorous or pleasant. The control did not differ significantly from the results for the left hemisphere.

We report here also the results of a study in which we obtained perimetric visual field data from subjects wearing the lenses. This was an experiment conducted on twelve subjects in which 'neuroticism' and 'extraversion' were studied in an attempt to find differences between the response on one hemisphere and that on the other. The Eysenck Personality Inventory was divided into alternate types of questions. These were printed out in large lettering so that they were easily visible, half were given to the left hemisphere and half to the right. The results of this experiment proved to be negative but more importantly we collected visual field data from each of the subjects. This was obtained in a darkened room using a standard perimeter on maximum size and illumination setting.

The subjects wore the contact lenses and their spectacles as previously described. The subjects placed their chin on the rest and were instructed to look straight ahead. The subject was asked to say "yes" when he saw the light appear and "no" when it disappeared. The direction and angle by which the light spot was brought into the subject's field of vision was randomised. The visual fields so obtained are shown in Fig. 2. It can be seen that vision is restricted to a small oval area occupying only a fraction of the visual field, located some 20° either to the left or right of central vision.

One striking incidental observation followed from this investigation after subjects removed the lenses at the conclusion of the experiment while still sitting at the perimeter apparatus. They were greatly surprised to find that they had not been stimulated at the centre of their vision. To them what they saw appears as part of subjective space undeniably central and certainly not as something displaced from the midline.

We report therefore a new phenomenon of 'centring' in vision, that is, the taking of small parts of vision displaced to one side and creating of it a realignment in experience such that it comes to occupy the centre of the experienced visual world. The brain not only takes a small restricted input and uses it to fill the whole span of visual experience, but so also does it take islands of vision displaced quite far to the periphery and make of them the centre point of the experienced visual world. The brain, in other words, creates its own centre of vision in spite of the lateral displacement of its image.

We suggest on the basis of these results that the two hemispheres of the brain of man possess an essentially different emotional vision of the world. The objective world although physically the same for each side, has a different perspective imparted to it. We suggest that the right hemisphere adds its own emotional dimension which represents the thing perceived as more unpleasant and horrible and thus aligns itself more with the characteristic perception of the depressive patient than with that of the normal individual. Whatever the nature of this form of perception, we believe that each hemisphere has its own

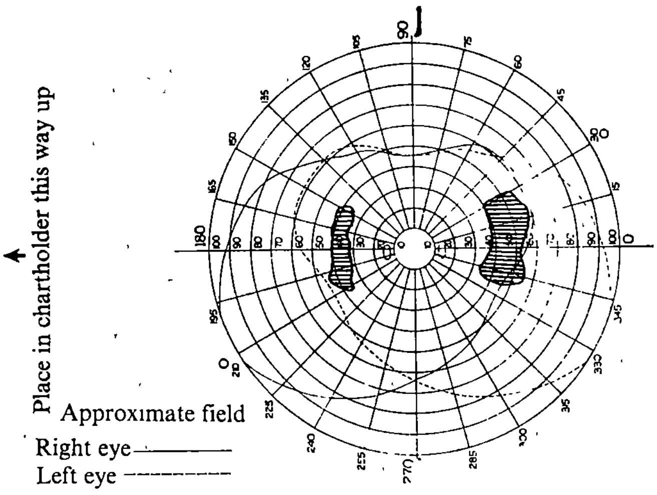

Fig. 2 Areas of vision for a typical subject wearing lenses for the right or left visual field.

distinct emotional vision of the world and that each makes a unique contribution to the whole. At the same time the left hemisphere scores so closely resembled those for the free vision condition where it may be assumed that both hemispheres are stimulated, to suggest that it is usually the left hemisphere perception which predominates. The extra dimension of the emotional focus of the right hemisphere seems to be suppressed in ordinary vision. How far the right hemisphere enters into the everyday emotional appraisal of the environment is a matter for debate. With regard to the question of the ultimate unity of emotional judgment our results suggest that the left takes precedence over the right but we cannot discount the possibility that the emotional vision of the right is used in ways which cannot as yet be specified.

There could be a parallel to ideas of unconscious determination of conduct in the view that the right hemisphere provides a source for unconscious motivation within the brain. The right hemisphere does not seem to be denied access to consciousness, however, but instead contributes an alternative voice to mental action at the conscious level.

It may be that the differences reported here are cognitive ones arrived at as the result of viewing the film through one hemisphere channel rather than another. It is hoped that our records of heart rate and electrodermal response recorded simultaneously while viewing will allow us to distinguish whether some fundamental difference of emotional functioning between the hemispheres is indicated, or whether these results can be explained on the basis of a cognitive assessment made during or after viewing has taken place.

In the initial report on the research using this technique[8] we suggested that lateralisation had been successfully achieved although we could not discount the possibility that some input may pass to the midline or beyond to the opposite hemifield. On the basis of the lateral differences observed in that study and the visual field data presented here we now feel more confident that the hemifield lateralisation has been successful and we believe the technique to be a particularly useful one in cerebral laterality research.

We also believe that these techniques will be important for the restriction of input to small parts of the visual field to study functions related to local parts of the retina.

We conclude therefore that there exists a different emotional vision between one hemisphere and the other in the normal human and that the right hemisphere has an emotional vision which is usually suppressed but is characterised as more unpleasant and horrific than is that of the left.

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Significance of two frequency bands in long distance vocal communication in the green treefrog

VOCAL communication is crucial in the reproductive biology of the green treefrog (*Hyla cinerea*). The male calls at night near a suitable breeding site: the acoustic signal serves for both localisation and species recognition by the female[1-3]. A typical mating call has two frequency bands of about the same relative amplitude: the lower one consists of one component of about 1 kHz; the upper one consists of one to four (usually two) components at about 2.7–3.3 kHz (ref. 2). Both frequency bands are pertinent for species discrimination at a moderate sound pressure level (SPL), corresponding to the SPL at 2–4 m from vocalising males[4,5]. This report discusses the significance of the two frequency bands in communication over greater distances. Not only does the SPL decrease with distance, but also the relative amplitudes of the two spectral peaks change because excess attenuation is frequency dependent. In general, high frequencies attenuate more rapidly than do low frequencies[6,7]. I found that a low frequency component of the call was more attractive to females than were high frequency components. Furthermore, in discrimination experiments females detected attenuation of a 3-kHz component relative to a 0.9-kHz component at moderate to high SPL but failed to do so at low SPL.

The minimum SPL necessary to attract gravid female treefrogs within 5 min from 1 m in front of a Nagra DH speaker is here defined as a behavioural threshold. Three kinds of tone bursts were presented: 0.9, 3.0 and 2.7+3.0 kHz (the two components had the same relative amplitude and were phaselocked). The sounds were recorded and played back with a Nagra S tape recorder. A Brüel and Kjaer 2209 sound level meter was used to adjust SPL (dB relative to 2×10^{-4} μbar r.m.s. 'fast'). Tests were carried out at night outdoors on a level cement driveway at air temperatures of 20–26 °C. Each of 34 females was collected in amplexus near Savannah, Georgia and tested individually at 1–4 SPL per stimulus (about 2 min between trials). Nine of the animals responded to two different sounds. The females had lower behavioural thresholds to the low fre-

Table 1 Responses of 34* female *H. cinerea* to synthetic calls of variable sound pressure level

Stimulus	Number tested	SPL (dB re 2×10^{-4} μbar)	Number of trials	Oriented to speaker	Approached speaker (within 50 cm)	Touched speaker
3.0 kHz	12	95	10	3	3	2
		90	11	2	0	0
2.7+3.0 kHz	12	77	8	6	5	5
		72	12	7	5	4
0.9 kHz	19	60	13	11	9	4
		55	9	5	2	0

*Nine females responded to two different sounds. Additional responses were observed at higher SPL than those included in the table. Lowest SPL which attracted females is considered to be the behavioural threshold for the stimulus used. Females failed to respond when the SPL was lowered by 5 dB. Only two of the four females which touched the speaker when 2.7+3.0 kHz was broadcast at 72 dB were tested at 67 dB. Call duration was 0.16 s and repetition rate was 0.8 s. See Gerhardt[4] for details of the synthesis of experimental stimuli.

quency call (0.9 kHz) than to the two-component (2.7+3.0 kHz) high frequency call (Table 1). The temporal patterning (300 s^{-1} beating) of the latter stimulus undoubtedly contributed to its lower threshold (Table 1) as compared with the 3-kHz stimulus[8]. Because of relatively high background noise levels, I consider these threshold determinations to be crude and conservative; nevertheless, I emphasise the significant differences in the relative attractiveness of the three sounds.

Neurophysiological studies of *H. cinerea* from Savannah are consistent with the above behavioural results. Moffat and Capranica[9] found two (of three) populations of neurones in the auditory nerve that seem to be directly involved in mating call processing: in one, neurones are most sensitive to frequencies at about 0.6–1.2 kHz; in the other, neurones are tuned to about 3.2–3.6 kHz. The low frequency units are, on the whole, much more sensitive than the high frequency units. Lombard and Straughan[10] obtained comparable results when they recorded evoked potentials from the midbrain of *H. cinerea* from Florida.

Besides indicating that low frequency components of the call will attract the female from a greater distance than high frequency components, the above results suggested that in call (species) recognition the relative importance of the two spectral peaks may depend on the SPL of the sounds. Accordingly, 78 females from Savannah were tested in discrimination experiments. Each animal was released midway between two Nagra speakers placed 4 m apart. Synthetic calls consisting of two phase-locked components of 0.9 and 3.0 kHz having the same relative amplitude were broadcast from one speaker; and calls with the same components having unequal relative amplitudes from the other. Calls were presented alternately every 0.4 s. I equalised the SPLs at the point where I released each female, and defined a response as a physical contact with a speaker. At a low playback level (65 dB) females discriminated against a synthetic call having an attenuated lower peak but failed to discriminate against a call with an attenuated upper peak (Table 2). At a high SPL (85 dB) females discriminated against the call with the attenuated upper peak but failed to discriminate against the call with attenuated lower peak. Note that 85 dB is above the behavioural threshold for a high frequency call with two components (2.7+3.0 kHz) but below the threshold for the 3-kHz call (Table 1). Because fine temporal as well as spectral patterns are pertinent, generalisation of the above discrimination results using synthetic calls with two high frequency components (0.9+2.7+3.0 kHz) will be important.

Table 2 Responses of 78* female *H. cinerea* in discrimination experiments

SPL (dB)	Number of females responding to: 0.9 kHz (0 dB) + 3.0 kHz (0 dB)‡	Number of females responding to: 0.9 kHz (−12 dB) + 3 0 kHz (0 dB)‡	Probability (Two-tailed binomial)
65†	13	1	0 002
70–75†	11	1	0.006
85+	14	7	Not significant
	0.9 kHz (0 dB) + 3.0 kHz (0 dB)	0.9 kHz (0 dB) + 3.0 kHz (−9 dB)	(Two-tailed binomial)
65	6	6	Not significant
70–75	8	2	0 080
85	9	0	0.004

Each female was used in only one test, and only first responses were tabulated. Duration of synthetic calls was 0.16 s and repetition rate was 0.8 s. See Gerhardt[4] for further details.

*Five of these females were used in threshold trials (Table 1) after they responded in a discrimination test.

†The highest modal SPL of the mating calls of *H. cinerea* measured in the field was 93 dB (RMS) at 0.5 m (ref. 5) If this is taken as a reference point and it is assumed that attenuation is solely due to spherical spreading, then these levels (60, 70–75 and 85 dB) correspond to distances of about 13, 7–4, and 1.3 m respectively from the sound source.

‡Relative amplitudes were measured (General Radio 1900-A wave analyser) from recordings (Nagra 4.2 recorder, Sennehiser 815 microphone) made 2 m from the speakers on a cement substrate.

In certain uniform conditions, distance attenuation from a point source increases monotonically with frequency[11]. Direct measurement by Morton[7], however, showed that in complex, natural environments frequency-dependent excess attenuation differs dramatically from habitat to habitat. Preliminary analyses (H.C.G., unpublished) of spectral changes in natural and synthetic frog calls after propagation through natural environments corroborated Morton's results. Furthermore, I found that the elevations of both the sound source and measuring point had a significant bearing on the attenuation of the high frequency relative to the low frequency components.

I suggest, therefore, that the low frequency components of the male's call attract the female at a distance. As she approaches the male (or chorus), high frequency components also become important for species recognition. The distance at which the high frequency components have sufficient energy to affect the female's behaviour will depend on the habitat and locations of the frogs.

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Rates of size increase and of phyletic evolution

THE RATE of phyletic evolution (evolution of the non-branching, non-cladogenetic type) is highly correlated inversely with the overall population size of genera and species. Factors which can materially decrease overall population size include increases in one or more of the following. number of biogeographic entities; number of communities; degree of climatic differentiation; and degree of marine regression on the continents (smaller populations of shallow water, marine organisms with regression). When all of these factors act in one direction rates of phyletic evolution are high or low; when they interfere with each other rates are intermediate[1,2].

An additional factor is size. Cope's Rule long ago formalised common knowledge that during evolutionary history many taxa tend to increase in size. Other taxa tend to decrease in size with time. Hallam's data[3], based on Jurassic bivalves and ammonites, indicates that genera and species showing an evolutionary (not environmentally) large rate of size increase have shorter time ranges than do taxa showing a small rate of size increase or no increase. Hallam's data, as well as those available from other time intervals, indicate that such size changes are nonlinear over long time intervals. Hallam[3] has used Fursich's data to show that larger Jurassic fossil taxa from one region are less numerous as individuals than is the case with smaller fossil taxa This last relationship is characteristic of living animals (see Reish[4] for a typical example).

Stanley[5] points out that most species of higher taxa (such as Class, Order, or Superfamily) are in the lower part of the total size range known for the higher taxon.

Figure 1 indicates the upper and lower size limits imposed on the species of higher taxa by their physiological, mechanical and ecological attributes as discussed by Stanley. He gives ecological and physiological explanations of why most species belonging to a higher taxon will tend to the lower rather than the upper size limit. Figure 1 indicates that many species will evolve phyletically with no change in size, whereas others will increase or decrease. Of the species changing their size, some will do so rapidly and others slowly. Stanley's Fig. 3 makes it clear that size increases will tend to affect a relatively small number of species belonging to any higher taxon.

Hallam's 'curves' (from his Fig. 2) for change in size of Jurassic molluscs with time can be interpreted as different segments of the curves of size change against time (Fig. 1) for forms undergoing rapid, slow or fluctuating changes in size. Consideration of Fig. 1 suggests that Hallam studied too small a sample through geological time to have been able to see more than a small, albeit significant, portion of the total size change curve for any taxon he considered.

The fossil record implies that most phyletic evolutionary changes in size have been moderate, since increasingly smaller percentages of species approach the upper and lower size limits known for each major group.

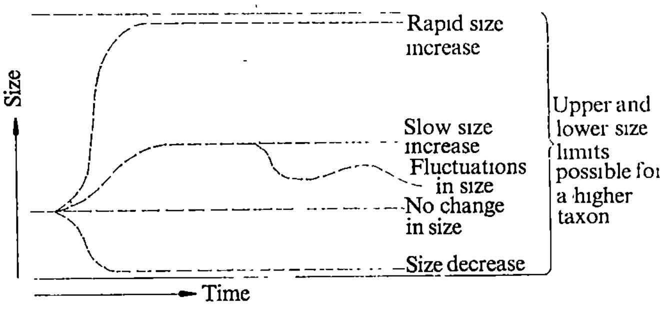
Consideration of sizes and size changes through time for fossil and recent organisms makes it reasonable to conclude that the various rates of size change are about equally likely for both increases and decreases. The data also suggest that lower rates of increase and decrease are more common than are higher rates.

Hallam also observed that ". . younger taxa are often appreciably smaller than their evolutionary precursors, but with no discernible size transition from one to the other." This conclusion follows (Fig. 1) because the modal size for any animal group is far closer to the lower than to the upper size limit characteristic of the group. The chances of sampling adequately the fossil record for the brief, downward size changes is less than for the more extended, upward size changes—with both changes occurring at the same rate. Consideration of the Silurian–Devonian brachiopod record accords with Hallam's observations about the absence of intergrades showing size decrease as opposed to the more extensive intergrading sample found for size increase.

Hallam's data[3], as well as Silurian–Devonian brachiopod data, strongly indicate that evolutionary changes in size occur at non-uniform rates. Hallam's observations are founded on intraspecific and intrageneric evolution in Jurassic molluscs whereas mine are based on intrageneric and intergeneric relations among Silurian–Devonian brachiopods Those groups of Silurian–Devonian brachiopods showing a significant size increase with time exhibit a nonlinear rate of increase. The rate of increase in size is a function of the magnitude of the increase, as also suggested by Hallam's data. For example, during the 20 Myr or so for which we have good information regarding widespread occurrences of the spiriferid *Howellella* the shells have a maximum width of about 1 cm, until near the end of the Silurian, when a small increase in size begins to be evident. By the end of the Lower Devonian (another 15 Myr or so) some of the descendants of the Silurian *Howellella* such as *Acrospirifer, Euryspirifer* and *Fimbrispirifer* reach 8 cm in width (a rate of only a few mdarwins during 20 Myr followed by an average rate of about 250 mdarwins for 15 Myr!). The increase from a little more than 1 cm to 8 cm or so occurs with increasing rapidity during the Lower Devonian. *Atrypa* of the *A "reticularis"* type have a maximum width during the Silurian of about 2 or 3 cm whereas in the Lower Devonian there is an increase to about 5 cm (no significant increase during the Silurian followed by an average rate of about 70 mdarwins during the Lower Devonian). This average rate of increase is far lower than that of the spiriferids, although it is significant. Taxa showing overall decrease in size are well known among such groups as the chonetids and stropheodontids during this same time interval, although in these groups far more show increases in size as predicted by Stanley's Fig. 3. Other genera such as *Skenidioides* and *Dicaelosia* show no significant increase in size during the Silurian–Lower Devonian time interval.

Palaeontologists have found that during some time intervals related species of diverse groups tend to be larger than during other time intervals. In North America Middle Ordovician brachiopods tend to be significantly smaller than those of the uppermost Ordovician (Newell[6] comments on this relationship). I have earlier[7] mentioned the fact that

Fig. 1 Diagrammatic representation of upper and lower size limits for species belonging to a major taxon, with an indication of taxa increasing and decreasing their size phyletically from the overall, lower size characteristic of the major taxon



Silurian brachiopods as a whole tend to be small relative to Lower Devonian brachiopods belonging to the same families and subfamilies (other groups such as bivalves show the same trend). Uppermost Devonian (Famennian)–Lower Carboniferous brachiopods are significantly smaller than those of the Upper Carboniferous–Permian. (Newell[6] supports this conclusion with data from other groups as well.) Nicol's comments[5] regarding the overall larger size reached by many Upper Cretaceous bivalves as contrasted with Lower Cretaceous and Jurassic ones is another good example. What explanation accounts for these non-random, time-correlated increases in size?

The more rapid the rate of phyletic evolution characteristic of a group the larger will be the number per unit time of species showing an increase in size, and the larger will be the number of species showing other evolutionary changes. Times of increasing overall rate of phyletic evolution for any major group of organisms will be the same time intervals when number, percentage and production rate of taxa increasing in size will be at a maximum. Conversely, the lower the overall rate of phyletic evolution characteristic of a group the smaller will be the number and percentage of taxa which achieve a larger size than is normal or modal for the group. It is noteworthy that an abrupt increase in phyletic rate commonly coincides initially with the brief upsurge in cladogenetic rate accompanying major provinciality increase[1].

A major terminal extinction event (such as those that occurred at the end of the Ordovician, the end of the Frasnian, the end of the Permian, and the end of the Cretaceous) will tend selectively to eliminate the larger, more specialised, more stenotopic species. There may then be a lengthy interval of relatively low overall rate of phyletic evolution, with few large species being produced, that is ultimately followed by an interval of higher overall rapid rate of phyletic evolution during which a higher number of large species is produced. Of course, there need be no long interval of low phyletic evolutionary rate and low number of large species. Silurian–Devonian brachiopods following the terminal Ordovician extinction event have a low overall rate of Silurian phyletic evolution with smaller number of large species, followed by a high rate of Lower Devonian phyletic evolution with large number and percentage of large species. The Permo–Carboniferous following the Late Devonian (terminal Frasnian) extinction event has a low rate of Famennian–Lower Carboniferous phyletic evolution with smaller number of large species followed by a high rate of Upper Carboniferous–Permian phyletic evolution with large number and percentage of large species. The Jurassic–Cretaceous data are similar, as detailed earlier.

For the neritic marine benthos considered here, which probably had planktotrophic larvae, there is no reason to think that numbers of larvae[9] will differ markedly between individuals of large and small size belonging to various species. But a far smaller absolute number of larvae belonging to species of larger size will attain maturity than is the case for species of small size. As regards mammals it is obvious that their reproductive characteristics dictate much smaller broods and lower reproductive rates in species of large size than in those of small size. This correlation of reproductive rate with size in mammals should help to exaggerate the disparity in phyletic rate between large and small mammals in terms of population size. Clearly, the critical factor relating to rate of evolution and population size for mammals large and small, as well as invertebrates, is the number of individuals reaching the reproductive stage.

In principle the low population densities characteristic of abyssal benthos would suggest rapid phyletic evolution, as does the possibility that a non-planktotrophic larval habit is widespread, but the level of cosmopolitanism characteristic of many abyssal species may counteract this possibility, and there is no suggestion that large species in most groups are more abundant in the abyss than on the shelf.

The relation between population size and taxon size (size of individuals in a taxon) also holds on land as indicated by Wolff's[10] recent compilation of information for mammals. There is also a tendency for large mammals to evolve more rapidly than small mammals such as rodents. Maglio's[11] work on the elephants and Hecht's[12] consideration of the evolution of the polar bear are both highly consistent with an inverse relation between population size and rate of evolution.

The speed with which infraspecific changes in size may occur in conditions of small population size is underscored by the work of Foster[13], Corbet[14], Berry[15], and Delany[16], all of whom provide evidence indicating that small populations isolated on small islands from a large mainland population depart from normal size very rapidly. It takes little imagination, post-Darwin, to combine this information with other data showing the uniqueness of island faunas in many parts of the world and then to see the high correlation between rates of evolution and population size.

Hallam's conclusion that larger taxa have a shorter time duration geologically than smaller ones follows from the premise that species of large size are generally more stenotopic and therefore more susceptible to terminal extinction when faced with significant environmental perturbations. Hallam's conclusion accords well with the fossil record of the marine invertebrates known to me, and would certainly seem to agree well with what is claimed for terrestrial vertebrates. In other words, there is a survival premium based on eurytopy that accompanies moderate size for the species of any major taxon. It must be emphasised here that the shorter time duration of rapidly evolving large species commonly involves terminal extinction whereas the shorter time duration of small population endemic species, many of which may also be of small or moderate size, involves phyletic evolution, or phyletic extinction, if you will. Small population endemic species of large size will be subject to rapid phyletic evolution for the same reasons as endemic small species but the large species will be more prone to terminal extinction due to any environmental perturbations.

In conclusion, it seems to me that the inverse correlation of the rate of phyletic evolution and size of interbreeding population is consistent (in addition to items considered earlier[1]) with the following.

(1) Evolutionary changes in animal size are chiefly nonlinear. (2) Increasing rate of phyletic evolution correlates positively with increasing size. (3) For any group of animals, or between groups of animals, the number of individuals of any species decreases as the average size of individuals increases. (4) The number and percentage of large species in any major group is larger during times of rapid phyletic evolution than during times of slower phyletic evolution. (5) The above evidence indicates that during times of rapid phyletic evolution there tends to be an increased number and percentage of species evolving which are larger sized and have small populations; this in turn helps to further speed up rate of phyletic evolution.

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Synergistic effect of a pheromone and a kairomone on host selection and colonisation by *Ips avulsus*

WE report for the first time a synergistic effect on the pheromone response of a bark beetle species (Coleoptera· Scolytidae) by the pheromone of a sympatric species. *Ips avulsus* Eichh. and *I. grandicollis* Eichh. are two sympatric *Ips* species often found colonising the same host tree in the southern USA. Male *I. avulsus* produce the pheromone ipsdienol (2-methyl-6-methylene-2, 7-octadiene-4-ol)[1,2] and both sexes are attracted to this compound in the field[3]. Male *I. grandicollis* produce the pheromone ipsenol (2-methyl-6-methylene-7-octene-4-ol)[1,2,4] and both sexes respond to the S-(−)-isomer[5]. *I avulsus* is known to respond in low numbers to both the crude attractant[6] and infested host material[7] of *I. grandicollis.* To investigate this relationship, a series of tests was carried out in east Texas. Two to four tree trunk-simulating olfactometers[8] set 10 m apart adjacent to a loblolly pine stand at Lufkin, Texas, were baited with test material during the afternoon, when flight of *I avulsus* occurred. Test materials included the optically pure enantiomers of ipsenol[9], racemic mixtures of ipsenol and ipsdienol, and billets infested with male *I. avulsus* and *I. grandicollis* All test materials used in a test series were offered simultaneously.

The results (Table 1) show that *I. avulsus* responded in significantly higher numbers to combinations of its own pheromone (ipsdienol) and that of *I. grandicollis* (ipsenol) than to either pheromone alone. This synergistic effect also occurred when natural attractants (infested billets) were used *in lieu* of synthetic pheromones. Furthermore, the active optical fraction of ipsenol is the S-(−)-isomer, the same isomer which causes aggregation of *I grandicollis*[5].

Most scolytids, including *I avulsus,* occupy temporary habitats; therefore, successful selection and colonisation of suitable host material is a critical phase in the life of these insects. The evolution of secondary attraction in order to fully exploit these habitats has been suggested[10] Moreover, the synergistic effect of a pheromone–kairomone interaction, where the latter is produced by a related species, seems to be an additional adaptive strategy favouring successful host colonisation.

The mechanisms responsible for reproductive isolation in *I. avulsus* and *I. grandicollis* are unknown, but are probably related to differences in habitat preference within the host tree, specificity of mating behaviour, and genital incompatibility. With reference to *I avulsus* and *I. grandicollis,* our findings[3] do not support the hypothesis that specificity of pheromones among sympatric *Ips* species is an important mechanism in maintaining species integrity and preventing interspecific competition[11,12], nor the more recent suggestion that sympatric species of *Ips* further reduce interspecific competition through mutual pheromone inhibition[13].

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Table 1 Field response of *I. avulsus* to olfactometers baited with synthetic and natural attractants

Test series 1975	Test material	Release system*	No. of repetitions	Length of repetitions (min)	Average number of beetles trapped		Sex ratio (male–female)
I (2 September)	Ipsdienol	a	4	25	3.75	a†	1.5:1
	Ipsdienol+ (+)–Ipsenol	a b	4	25	5.00	a	1 9:1
	Ipsdienol+ (−)–Ipsenol	a b	4	25	34.75	b	2.9:1
	Ipsdienol+ Ipsenol	a	4	25	22.50	b	2.8:1
II (11 September)	Ipsdienol	a	4	45	2.75	a	4.5:1
	(−)–Ipsenol	b	4	45	8.00	a	3.6:1
	Ipsdienol+ (−)–Ipsenol	a b	4	45	7.25	a	8.7:1
III (11 September)	Ipsdienol	a	5	40	9.40	a	3.3:1
	Ipsdienol+Ipsenol	a	5	40	25.60	a	3.9:1
IV (18–19 September)	Ipsdienol	a	13	15	5.23	a	0.84:1
	Ipsdienol+Ipsenol	a	13	15	19.00	b	2.4:1
V (7–9 September)	*I. grandicollis* billet w 25 ♀ ♀+uninfested billet		5	60	2.00	a	9.0:1
	I. avulsus billet ⓦ 25 ♀ ♀+uninfested billet		5	60	8.20	a	2.2:1
	I avulsus billet and *I. grandicollis* billet, each ⓦ 25 ♀ ♀		5	60	17.00	b	3.5:1
	Ipsdienol+Ipsenol	a	5	60	18.20	b	3.0:1

*a, Vial: 5 mm internal diameter × 30 mm; b, Capillary: 0.9 mm internal diameter × 10 mm.
†Means with different letters are significantly different at $P<0.05$.

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Evidence that blackfly larvae can feed on particles of colloidal size

FILTRATION of colloidal particles has not previously been reported for animals living in flowing freshwaters. The observation that blackfly larvae (Diptera : Simuliidae) can filter such particles may alter our understanding of the nutrition of these suspension-feeding animals and also afford new possibilities for the control of the insect in areas where the adult females are vectors of onchocerciasis.

Blackfly larvae are equipped with specialised mouthparts, the cephalic fans, which, when extended into the current, trap suspended particles. At intervals the fans are folded and particles trapped on the fans are removed by the mandibles, passed to the cibarium and, thence, to the foregut. Recorded lengths of ingested particles vary between 0.5 and 300 μm with the majority in the range 20–100 μm for some Canadian larvae[1], and from 11.3 ± 1.8 to 15.1 ± 2.0 μm for several British species[2]. That larvae can feed exclusively on fine particles has been demonstrated by rearing them to pupation on a diet of *Bacillus subtilis* (Ehr.) Cohn in distilled water[3].

In the present investigation an aquarium tank ($13 \times 13 \times$ 17 cm) was filled with distilled water and a circulating current created by an air pump. A concentrated suspension of polystyrene latex microspheres (Dow Chemicals) of diameter 0.091 ± 0.0058 μm was added to the water to give a suspension of less than 0.02% by volume Large larvae (fifth instars and above, mainly of *Simulium nitidifrons* Edwards) were collected from a stream and placed in the aquarium for 24 h after which they were removed and killed in formalin.

The larvae were then cut into thick sections ($\sim$1 mm) using a scalpel and the surface of each viewed in a scanning electron microscope. In section, the gut contents were white and granular When a little of this material was observed by transmission electron microscopy it was seen to consist of dense, three-dimensional aggregates often composed of many hundreds of latex microspheres.

Aggregations of microspheres were rarely observed in samples from the concentrated, or the dilute experimental suspension. Those aggregates which were observed consisted of few microspheres arranged in a single layer and probably resulted from drying the suspension on grids before examination by transmission electron microscopy It is therefore concluded that the large three-dimensional aggregates found in the gut must form either on the cephalic fan rays, or as the result of 'packing' after the ingestion of single spheres.

It thus seems that blackfly larvae are able to feed on particles as small as 0.091 μm in diameter although the details of the feeding mechanism have yet to be fully understood. The use of particulate insecticides of 10 μm diameter against blackfly larvae has been demonstrated to be selective[4]. An insecticide preparation of smaller dimension would reduce sedimentation and allow the treatment to be even more selective.

The nutrition of blackfly larvae has not been studied in detail but fine particles, in addition to larger ones, may form an important part of the diet[1] In 1909 it was suggested that dissolved organic matter was utilised in the nutrition of some aquatic animals[5], a theory which has subsequently been questioned. In the light of the present findings this theory may well have some basis in the case of blackfly larvae.

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Prochlorophyta as a proposed new division of algae

UNICELLULAR algae associated with ascidians from tropical Pacific shores have been reported by various biologists[1–5]. They are bright green, generally spherical and about 10–20 μm in diameter, and they seem to have no clearly delimited nucleus or plastids. Such cells (identified as *Synechocystis didemni*), found associated with surfaces of *Didemnum* colonies on the Pacific coast of Mexico, have been shown by electron microscopy to be prokaryotic[6,7], which suggests that they are cyanophytes, that is, blue-green algae. Although all known blue-green algae (other than a few apochlorotic types) contain phycoerythrin, phycocyanin, or both, however, these ascidian symbionts are apple green and contain no detectable bilin pigments. Furthermore, like the eukaryotic algae in the divisions Chlorophyta and Euglenophyta, they contain two chlorophyll components, separable by chromatography and provisionally identifiable as chlorophylls *a* and *b* (ref. 8), whereas no cyanophytes are known to contain chlorophyll *b*. The assignment of *S didemni* to any of the established algal divisions, therefore, presents a major taxonomic problem.

Algal cells found living inside the colonies of other didemnid ascidians at Enewetak Atoll, Marshall Islands, and on Coconut Island, Hawaii, have been recently studied by the same techniques and have proved to be very much like those of *S didemni*. My colleagues and I (unpublished results) have confirmed in particular that those from *Diplosoma virens* (from Hawaii) are prokaryotic, contain chlorophylls *a* and *b*, contain no detectable bilin pigments (even after incubation for one or more days in nitrogenous media) and show no evidence of phycobilisomes on the thylakoids. In the light, in aerobic conditions, they can fix CO_2 and evolve oxygen. They must therefore be considered as algae (rather than as photosynthetic bacteria, which are normally anaerobic and, when illuminated, fix CO_2 without concomitant oxygen evolution). They cannot, however, be logically assigned to the Cyanophyta, the Chlorophyta, the Euglenophyta, or any other existing division of the algae.

In view of the fact that their peculiar combination of characteristics is common to algal symbionts from several different ascidians found in widely separated locations, it now seems appropriate to put such organisms in a new algal division, which I propose to call the Prochlorophyta. Since it will consequently be necessary to remove the type species, *S didemni*, from the Cyanophyta, it can no longer be retained in the blue-green algal genus *Synechocystis*, in spite of morphological similarities. The new division is formally described in Latin (requisite for all new plant taxa) as follows: *Prochlorophyta: algae procarioticae similes Cyanophytis, cuiis differentes pro formatio chlorophyllarum binarum (a, b-que); pigmentia rutila aut caerulea absunt.* (Formal descriptions of the new class, order, family, genus and type species will be presented elsewhere.)

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Chemotherapy of *Theileria parva* infection

EAST coast fever (ECF), the most important tick-borne disease of cattle in eastern and central Africa, is caused by the protozoan parasite *Theileria parva*. No successful therapy is available and mortality can reach 96% (ref. 1), although large doses of tetracyclines have some prophylactic effect[2]. The search for an active drug has been hindered by the lack of a laboratory model of the infection. An *in vitro* screening process has been established, using the infected bovine lymphoid cell culture methods developed by Malmquist *et al.*[3]. This has now shown that 2-hydroxy-3-(8-cyclohexyloctyl)-1,4-naphthoquinone (Menoctone, Sterling-Winthrop) has a high level of anti-theileria activity *in vitro*. Subsequent tests in cattle artificially infected with ECF confirmed the activity of this compound against the disease.

Screening was conducted with the E174 line of *T. parva*-infected bovine lymphoid cells grown in glass, screw-cap tubes, in the supplemented Eagle's minimum essential medium described by Malmquist *et al.*[3]. The tubes were 'gassed' with a mixture of 5% carbon dioxide in air. Compounds under study were dissolved or suspended in a minimum volume of ethanol, then appropriately diluted with sterile culture medium before addition to the cultured, infected cells. Each test was duplicated. At the beginning of a test the cultures contained 3×10^5 cells ml^{-1}. Tubes were incubated for 48 h at 37 °C, with gentle agitation. The growth of untreated cultures was determined using a haemocytometer, and the growth of drug-treated cultures was estimated with reference to this value, using a five-point scale. Control cultures usually contained 8×10^5–10×10^5 cells ml^{-1} after 48 h, and this was scored 5^+. A tube in which there had been no increase in cell numbers was scored 3^+, and one in which all cells were dead was scored 1^+. This gave an indication of the toxicity to the host cells of the compounds being tested. To assay the effect on the parasite the tubes were centrifuged at 500*g* for 5 min, and a smear was made from the pellet on a glass microscope slide. This was stained by the method of Field and Sandosham[4] and the percentage of infected cells was counted.

A wide range of anti-parasitic compounds was tested *in vitro*, including several which had been reported (without confirmation) to show activity against ECF *in vivo*. The results are summarised in Table 1.

Only Menoctone showed appreciable activity, and was selected for further testing in cattle infected with ECF. Fourteen high grade Jersey heifers, weighing about 90 kg, were divided randomly into two groups of seven. They were infected with *T. parva* (Muguga) by injection of 1 ml of a stabilate prepared from ground up, infected ticks[5], *Rhipicephalus appendiculatus*, in front of the right ear. The course of subsequent infection was monitored by reading rectal temperatures daily, and by examination of Giemsa-stained smears of biopsy material, from the local drainage lymph node and the contralateral prescapular lymph node, for the presence of macroschizonts and microschizonts. Stained blood smears were examined for the presence of piroplasms in the erythrocytes.

Menoctone was formulated as a 10% solution in a mixture of 20% dimethylsulphoxide and 80% corn oil. The compound was injected intravenously into one group of seven cattle, while the remaining seven were kept as untreated controls. The first dose (5 mg kg^{-1}) of Menoctone was injected on the first day on which a temperature of 39.5 °C was recorded and macroschizonts were detected in the local drainage lymph node. A further five daily doses of 1 mg kg^{-1} were given, making a total dose of 10 mg kg^{-1}.

In the untreated cattle the typical ECF syndrome developed, and six died. The seventh became severely ill but recovered. In all seven treated with Menoctone the progress of the disease was soon halted; temperatures returned to normal levels and schizonts could not be detected in lymph-node smears. Within 48 h of the first injection of Menoctone, schizonts seemed to be degenerating and were apparently destroyed within the lymphoid cells. An animal was regarded as 'recovered' 3 d after schizonts were last observed in lymph-node smears. The results are summarised in Table 2.

On day 38 after their original infection, all eight surviving cattle were challenged with a similar injection of stabilate. All were highly resistant, although small numbers of schizonts were detected in the one surviving control and in two of the animals treated with Menoctone. Six normal cattle which received the challenge dose reacted severely and died.

Menoctone therefore seems to have a high level of activity against *T. parva* in artificially infected cattle, when injected after the infection has become established. In an earlier experiment Menoctone was injected on the day of infection at 5 or 2.5 mg kg^{-1} and on the subsequent 9 d at 1.0 or 0.5 mg kg^{-1}, a total of 14 or 7 mg kg^{-1}. In groups of five cattle infected with *T. parva* stabilate the disease was almost totally suppressed by these treatments, and no cattle

Table 1 Activity of compounds against *Theileria parva in vitro*

	Concentration (mg ml^{-1})	Cell growth	% Cells infected
Menoctone	1.0	4^+	22
	0.1	4^+	24
	0.01	5^+	44
	0.001	5^+	72
	0.0001	5^+	83
Chloroquine	1.0	5^+	97
Mepacrine	1.0	5^+	90
Pamaquine	10.0	4^+	96
Primaquine	10.0	2^+	98
Cycloguanil	1.0	4^+	97
Pyrimethamine	10.0	2^+	100
Amprolium	10.0	5^+	100
Decoquinate	10.0	5^+	100
Diaveridine	10.0	2^+	100
Clopidol	10.0	5^+	98
Metronidazole	10.0	5^+	97
Robenidene	10.0	5^+	88
Sulphaquinoxaline	10.0	5^+	88
Amicarbalide	10.0	5^+	81
Imidocarb	100.0	3^+	97
Phenamidine	10.0	5^+	85
Diminazine diaceturate	100.0	3^+	100
Suramin	10.0	5^+	88
Oxytetracycline	100.0	4^+	97
Ethanol	(0.01%)	5^+	97
None	—	5^+	98

Table 2 Activity of Menoctone against *T. parva* in cattle

	Macroschizonts	Temperature 39.5 °C	Days to: Recovery	Days to: Death
Treated (0/7 died)	9.0±0.0	7.7±1.7	18.4±5.0	—
Untreated (6/7 died)	8.8±0.4	8.8±1.3	27	18.5±2.4

The total dose of Menoctone was 10 mg kg^{-1}. Figures show group means and standard errors.

became clinically ill Three of five controls died, and a fourth was severely affected. The fifth developed only mild ECF. On challenge, the cattle dosed with Menoctone at 14 mg kg^{-1} were highly susceptible, while those dosed with 7 mg kg^{-1} were partially resistant, suggesting that the drug acted very early in the course of infection, before immunity had become established.

The mode of action of Menoctone on *T. parva* is not clear, although it seems to act against the schizont and possibly earlier stages of the cycle. Peters[6] suggested that the antimalarial activity of Menoctone may be due to a blocking of the biosynthesis of coenzyme. The apparent destruction of the *T. parva* schizont within the host lymphoid cells, both *in vitro* and *in vivo*, agrees with this suggestion. The number of different parasites against which Menoctone is active, which includes coccidia[7], and *Theileria annulata in vitro* (N.M., unpublished results) indicates action on a fundamental and widely occurring biochemical pathway. Of the 20 anti-parasitic compounds tested against *T. parva in vitro*, only Menoctone was active at levels not toxic to the host cells This suggests that the metabolism of *T parva* is closely adapted to that of the host cell. The difficulty of finding a compound which will exploit differences between the metabolism of the parasite and its host cell perhaps indicates why no effective therapeutic compound is available for this economically very important disease.

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Drug-containing lipid vesicles render drug-resistant tumour cells sensitive to actinomycin D

DEVELOPMENT of cellular resistance to actinomycin D (AD) has been reported in tumour cell populations *in vivo*[1,2] and *in vitro*[3–6]. Such cells have a lower capacity than normal to incorporate AD[3,6–8], prompting suggestions that the AD-resistant phenotype is determined by changes in plasma membrane function that reduce cellular permeability to this drug. Support for this proposed mechanism of drug resistance has been provided by experiments showing that treatment of AD-resistant cells with membrane-active detergents, to increase plasma membrane permeability, enhances AD uptake by resistant cells and reduces the concentration of AD required to inhibit cell growth[8,9]. This approach, while instructive, has the disadvantage that the detergent causes significant alteration in cell viability and also impairs cell growth[8]. We now report a new method for enhancing drug uptake by permeability restriction, drug-resistant cells which uses lipid vesicles as carrier agents to introduce AD directly into cells. As reported elsewhere[10,11], negatively-charged lipid vesicles composed of lipids or lipid mixtures that are "fluid" at 37 °C are incorporated into cultured cells by fusion with the cellular plasma membrane. Entrapment of drugs inside such vesicles and subsequent fusion of the vesicles with cells offers a potential method for bypassing the plasma membrane transport barrier to introduce drugs directly into the intracellular compartment. Since vesicles are not cytotoxic[10–12], their use as drug carriers allows direct evaluation of the cellular response to the introduced drug, free from the complications[8,9,13] associated with the use of detergents. The results presented here indicate that uptake of vesicles containing AD by AD-resistant cells produces inhibition of cellular RNA synthesis and cell growth at AD concentrations that have no effect on cells when added as free drug to the culture medium.

The AD-resistant Chinese hamster cell line, DC3F–ADX, (provided by Dr J. Biedler, Sloan–Kettering Memorial Institute) was grown in Eagle's MEM supplemented with 10% foetal calf serum. The isolation, characterisation and properties of this cell line have been described elsewhere[4,7]. Cells were grown routinely in MEM containing AD at 10 µg ml^{-1} to prevent emergence of AD-sensitive variants, but grown in medium without AD for 10–15 d before use in experiments. Vesicles were prepared from a lipid mixture containing 1 µmol of phosphatidylserine, 9 µmol of phosphatidylcholine and 10 µmol of cholesterol which were mixed in choloroform, evaporated to dryness in a vacuum and suspended in 1.0 ml of calcium- and magnesium-free phosphate-buffered saline (PBS) containing different concentrations of AD. This mixture was used since vesicles of this composition are incorporated into cells by fusion with the plasma membrane[10,11]. All lipids were purified and characterised in our laboratory and were chromatographically pure[14]. Trace amounts of ^{3}H-AD (Amersham Searle, 7 Ci mmol^{-1}) were added to the lipid mixture before evaporation as a marker for quantitating drug capture inside vesicles. The suspension of lipids and AD in PBS was mixed vigorously on a Vortex shaker for 10 min at 37 °C after which the preparation was sonicated in a bath type sonicator for 1 h at 37 °C under nitrogen to produce unilamellar vesicles[14]. The sonicated preparation was equilibrated for 30 min at room temperature and then passed through a Sephadex G-50 column to separate vesicles containing AD from untrapped AD. Capture of AD by vesicles was then determined by the extent of recovery of ^{3}H-AD in association with vesicles as a fraction of the ^{3}H-AD present in the original lipid suspension in buffer When

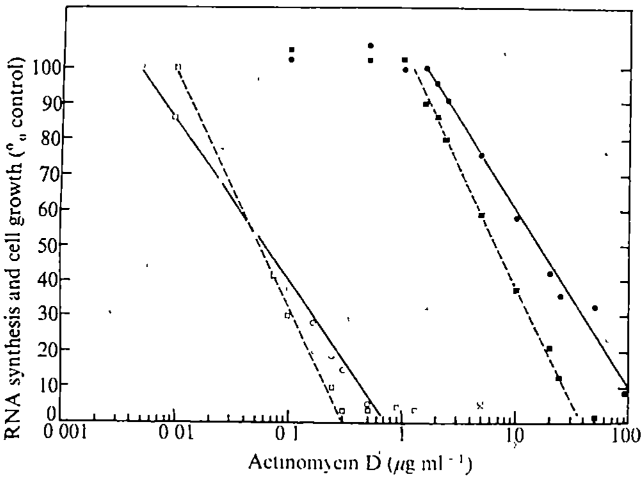

Fig. 1 Effect of free AD and AD entrapped within lipid vesicles on cellular RNA synthesis (———) and growth (- - - - -) of AD-resistant DC3F–ADX hamster cells. Closed symbols represent cells treated with free AD and open symbols represent cells treated with lipid vesicles containing different concentrations of AD. Exponentially growing cell populations cultured in 60-mm plastic Petri dishes (cell density 20×10^4 cm^{-2}) were incubated for 2 h at 37 °C in MEM containing either free AD at the indicated concentrations or lipid vesicles (200 nmol of lipid per 10^6 cells) containing different concentrations of AD. Vesicles were prepared as described in the text. The cultures were then washed with PBS to remove the drug or drug-containing vesicles and the ability of the cells to synthesise RNA and to proliferate was determined. Control cell cultures were incubated with drug-free medium for 2 h at 37 °C (see text for additional control experiments). To measure cellular RNA synthesis the drug-treated cultures were incubated in fresh medium containing ^{14}C-uridine (1.5 μCi ml^{-1}) and the amount of cell-associated radioactivity incorporated into a trichloracetic acid-insoluble fraction was measured after 17 h and expressed as a percentage of the incorporation occurring in replicate control cultures not exposed to AD. The ability of drug-treated cells to proliferate during 24 h was determined by the growth assay described previously[9] and the results were expressed as a percentage of the growth occurring in untreated control cultures. Each point represents a mean value from measurements of four cultures at the indicated drug concentration.

the starting AD concentration in buffer was 600 μg ml^{-1}, approximately 10% was captured by the vesicles. The efficiency of drug capture increased at lower starting concentrations of AD, so that at a starting dose of 17 μg ml^{-1} or lower, approximately 60% of available drug was trapped by the vesicles. This indicates considerable binding of the drug to the vesicles in addition to passive capture within the aqueous interior of the vesicle, which can be calculated as approximately 2% of the total. After separation of the vesicles on Sephadex G-50, the lipid concentration was 2 μmol of phosphate per ml with an incorporated AD concentration of 12 μg ml^{-1} (5×10^{-5} M) or less as indicated below. For comparison of cellular sensitivity to free AD and AD entrapped within vesicles, free AD was obtained from the effluent of the same column of Sephadex G-50 as the vesicles and diluted to the same concentration as vesicle-entrapped AD by dilution in PBS to give identical ^{3}H-AD counts. The free drug elutes as a delayed peak completely separate from the lipid peak.

Previous work has shown that the present DC3F–ADX cell line is highly resistant to AD, requiring 250 times more AD to produce effective inhibiton (>75%) of cellular RNA synthesis than the wild-type sensitive cell line (DC3F) from which it was derived[4,7]. Even when cultured in MEM containing AD at 50 μg ml^{-1}, limited RNA synthesis is still detectable in resistant cell populations (Fig. 1). But treatment of replicate populations of resistant cells with lipid vesicles containing different concentrations of AD resulted in effective inhibition (>75%) of cellular RNA synthesis at a drug concentration of 0.25 μg ml^{-1} (Fig. 1), a 200-fold decrease over the concentration of free drug required to produce the same effect. Measurement of the incorporation of ^{3}H-AD into resistant cells revealed that cells treated with vesicles containing ^{3}H-AD incorporated five to six times more AD than replicate cultures exposed to identical concentrations of free ^{3}H-AD (results not shown).

Enhancement of AD uptake into drug-resistant cells by vesicles also produced a significant reduction in the concentration of AD required to inhibit cell proliferation (Fig. 1). The concentration of drug required to reduce cell growth to 50% of that occurring in untreated control cultures was reduced from 7.2 μg ml^{-1} for cells exposed to free AD to 0.062 μg ml^{-1} for cells treated with vesicles containing AD (Fig. 1).

Control experiments in which resistant DC3F–ADX cells were incubated with similar numbers of vesicles without AD established that vesicle uptake *per se* had no effect on cellular RNA synthesis or cell growth (not shown). Further experiments in which resistant cells were incubated with vesicles without entrapped AD, but in medium containing free AD, also failed to alter cellular RNA synthesis or cell growth from that seen with free AD alone (results not shown). This suggests that vesicle uptake by cells does not alter significantly the transfer of free AD across the cellular plasma membrane.

The results in Fig. 1 thus indicate that vesicle-mediated enhancement of drug uptake by resistant DC3F–ADX cells substantially increases cellular sensitivity to AD These findings support previous proposals[3,5,7–9] that the AD-resistant phenotype results from a decreased cellular capacity to incorporate AD, produced by a reduction in the permeability of the cellular plasma membrane to this drug. But the fact that vesicle-mediated delivery of AD increases the uptake of AD five- to sixfold over that achieved with free drug, and yet is accompanied by a 200-fold reduction in the drug concentration needed to inhibit cell growth and RNA synthesis, raises the question of whether the vesicles deliver AD to a specially sensitive site of drug action within the cell. For example, AD binds to lipid-rich cellular components and the delivery of AD by the vesicle route might bypass some of this nonspecific binding.

The potential value of lipid vesicles as carriers for introducing pharmacological agents into cells has been proposed before[14–18]. Although our experiments were restricted to AD-resistant tumour cells, there is no *a priori* reason why lipid vesicles could not be used similarly to enhance the uptake of other cytostatic and cytotoxic drugs by resistant tumour cells. Resistance of mammalian tumour cells to methotrexate, daunomycin, adriamycin, *Vinca* alkaloids and the terephthalanides and related agents is believed to have a basis similar to AD resistance in that resistant cells fail to incorporate sufficient of these drugs due to reduced drug transfer across the plasma membrane[20].

Recent studies in this laboratory have also shown that vesicles can be used as drug carriers to increase the cytotoxic potency of nucleoside and nucleotide analogues. Ara-CTP, which is not ordinarily taken up by cells, has been introduced into tumour cells using vesicles as drug carriers with a significant increase in cytotoxic activity compared with ara-CTP alone (unpublished results of E. Mayhew, D. P., Y. M. Rustum, C. Dave).

Vesicle-mediated delivery of drugs into drug-resistant tumour cells might therefore provide a possible novel approach for cancer chemotherapy *in vivo*, particularly if "recognition ligands" could be included in the vesicle membrane to induce preferential uptake of vesicles by tumour cells Some initial progress in this direction has already been achieved *in vitro*, where incorporation of cell-specific immunoglobulins into vesicle membranes resulted production. Medium composition, aggregation state of cells, culture *p*H and cell density were all considered more important in determining glycolytic activity than growth rate. It should be noted, however, that these factors are also important to the proliferative rate of these cells. We believe that the important question is not whether culture conditions can influence lactate production, but rather whether glycolysis is linked to cell division.

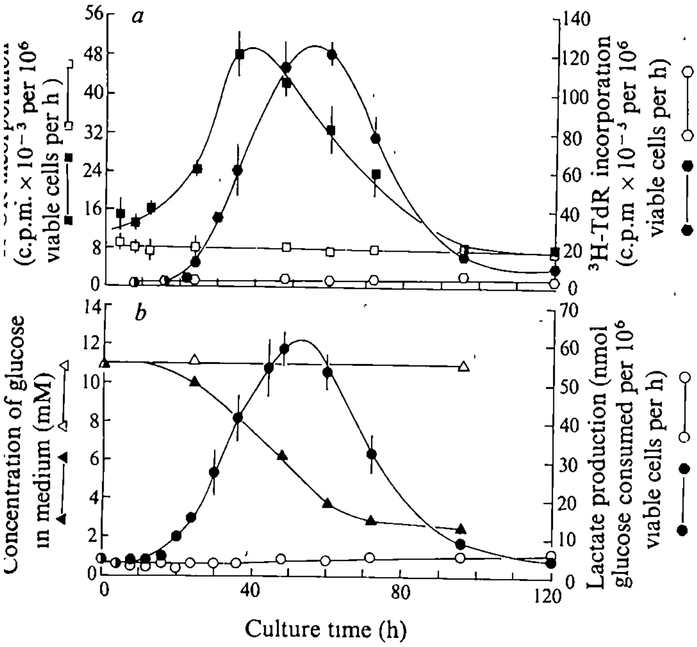

Fig. 1 The temporal relationship of macromolecular synthesis to aerobic glycolysis and glucose utilisation in mouse lymphocytes Cells collected from mouse (C57BL/6 × DBA/2F$_1$ hybrids, 8–12 weeks old from Jackson Laboratory) spleens by gentle homogenisation were treated with 0.87%' NH$_4$Cl to lyse erythrocytes. The resulting cell suspensions were 94–97% small lymphocytes by morphology The cells were washed and resuspended in RPMI 1640 medium with 2% pooled human serum, 2 mM L-glutamine, penicillin (100 U ml^{-1}) streptomycin (100 mg ml^{-1}). The cell density was adjusted to 5 × 10^6 cells per ml and 25 ml samples were placed in plastic Petri dishes (100 × 15 mm) and incubated at 37°C under 5% CO$_2$ in a humidified National incubator Mitogenic stimulation was produced by con A (2 μg per ml of culture) Viability was determined by Trypan blue exclusion. The closed symbols represent con A-stimulated cells and the open symbols control cells. The vertical bars at the individual time points represent 1 s.e m. For those points without the bars, the s e.m is smaller than the vertical dimension of the symbols. *a*, DNA synthesis (●, ○) was assayed by the incorporation of 0.5 μCi (6 Ci mmol^{-1}) ^{3}H-thymidine (^{3}H-TdR), RNA synthesis (■, □) by the incorporation of 0.5 μCi (6 Ci mmol^{-1}) ^{3}H-uridine (^{3}H-UR), and protein synthesis (not depicted because the curve is superimposable on the RNA curve) by 1 μCi (59 Ci mmol^{-1}) ^{3}H-leucine into trichloroacetic acid (TCA)-precipitable material For these labelling studies, at selected time points, 0.2-ml samples of the cell suspensions were transferred to microculture plates and labelled for 1 h The assays were terminated using a microculture harvester, the cells were precipitated with 5% TCA, deposited on glass fibre filters presoaked with the appropriate unlabelled precursors and washed After drying, the filters were immersed in scintillation fluid and counted in a Beckman L-230 liquid scintillation counter at a 30% counting efficiency. Each point is compiled from the means of triplicate determinations from four representative experiments. *b*, Lactate production (●, ○) was assayed at selected culture time points on cells washed and resuspended in glucose-free RPMI 1640 medium Duplicate 0.5-ml samples of washed cells were incubated for 30 min at 37°C in 5 mM ^{14}C-glucose (80 μCi mmol^{-1}), centrifuged and 0.4 ml of the supernatant was added to 0.5 ml 20% CuSO$_4$ 5H,O followed by 2 ml of H$_2$O and sufficient Ca(OH)$_2$ to form a bright, blue, fluffy precipitate. After 30 min at 25°C, the mixture was centrifuged and 1.8 ml of the supernatant way taken for a second CuSO$_4$/Ca (OH)$_2$ treatment Supernatant (1 ml) from the second treatment was mixed with 5 ml of scintillation fluid containing Triton X-100 and counted at 85% efficiency. Glucose concentrations (▲, △) were determined colorimetrically Duplicate 1-ml samples of culture medium or glucose standards were added to 1 ml of 5% phenol and 5 ml of concentrated, H$_2$SO$_4$ The absorbance at 490 nm was read in a Beckman spectrophotometer 30 min later.

So far no systematic study of the relationship of the appearance of aerobic glycolysis to cell proliferation or the phases of the cell cycle has been reported. This report presents evidence in normal lymphocytes that aerobic glycolysis not only is associated with cellular proliferation, but more specifically is temporally related to DNA synthesis.

Lymphocytes enter a proliferative cycle after stimulation by concanavalin A (con A). We have found, as have others previously, that among the early changes, the rates of protein and RNA synthesis were increased within 2–4 h (Fig. 1*a*)[14]. The increase in RNA and protein synthesis continued and both reached a maximum about 40 h after con A addition and decreased thereafter. DNA synthesis, on the other hand, did not appear until about 20 h and peaked between 50 and 60 h after stimulation (Fig. 1*a*). After this maximum, DNA synthesis fell and returned to baseline level. It is interesting that the decline in DNA synthesis, as well as RNA and protein synthesis, could not be prevented by frequent changes of medium, serum replenishment or the continued presence of con A. Thus, these cells undergo a discrete proliferative response (one to two cell divisions) to stimulation by con A and, unlike established lines or tumour cells, retain control of their division.

Glycolytic activity in lymphocytes during the proliferative response was assessed by measuring the production of ^{14}C-lactate from ^{14}C-glucose at selected times after stimulation by con A (ref. 15) Previous experiments had established that the rate of ^{14}C-lactate production was linear with pulse times up to 1 h and reached a plateau at a glucose concentration of 2 mM. When compared with a direct determination of lactate accumulated, this assay has the advantages of short measurement intervals, the elimination of background accumulation in the medium and the reduction of lactate reutilisation. This assay does, however, depend on the utilisation of exogenous glucose. Other sources, for example glycogen, might provide glucose for glycolysis. To investigate this possibility, the lactate released into the medium was measured directly by the lactate dehydrogenase assay[16]. The problems inherent in this method include a longer sampling time and presumed increased reutilisation. Nevertheless, the results of these determinations closely approximated those obtained with the ^{14}C-lactate assay (Fig. 1*b*) and only the latter will be discussed.

Lactate production changed considerably during lymphocyte proliferation and from the data presented (Fig. 1*a* and *b*) it can be seen that for the first 20 h of stimulation by con A, there was a small but significant increase in lactate production. With the onset of DNA synthesis at approximately 20 h, however, it increased sharply. Aerobic glycolysis reached a maximum value about 20 times greater than that of control cells by 50–60 h, then fell as DNA synthesis declined. Although these cells were not synchronised, it has been shown frequently that the amount of ^{3}H-thymidine incorporation correlates with the percentage of cells synthesising DNA[17]. The lactate production curve, in turn, coincides temporally with that of ^{3}H-thymidine incorporation, suggesting that it is the cells in S phase that generate most of the lactate. Furthermore, it should be noted that in these studies the cells progress through G$_0$, G$_1$ and S without being subjected to the artefacts caused by synchronisation techniques. Whether or not the intense aerobic glycolysis which accompanies DNA synthesis continues into G$_2$ and M, however, cannot be determined without cell synchronisation

The increase in glycolytic activity would be expected to be accompanied by a greater glucose consumption. Figure 1*b* shows that lymphocytes stimulated by con A before DNA synthesis utilised glucose slowly, but with the onset of DNA synthesis the rate sharply increased. Glucose consumption subsequently tapered off as ^{3}H-thymidine incorporation diminished, although concentration of glucose in the medium was still above saturation level (2 mM) for lactate production. There was no substrate limitation for glycolysis in these culture conditions, and in other experiments replenishment of the glucose to the original concentration (11 mM) neither increased glucose utilisation measurably nor prolonged DNA synthesis.

The close correlation of DNA synthesis and aerobic glycolysis is

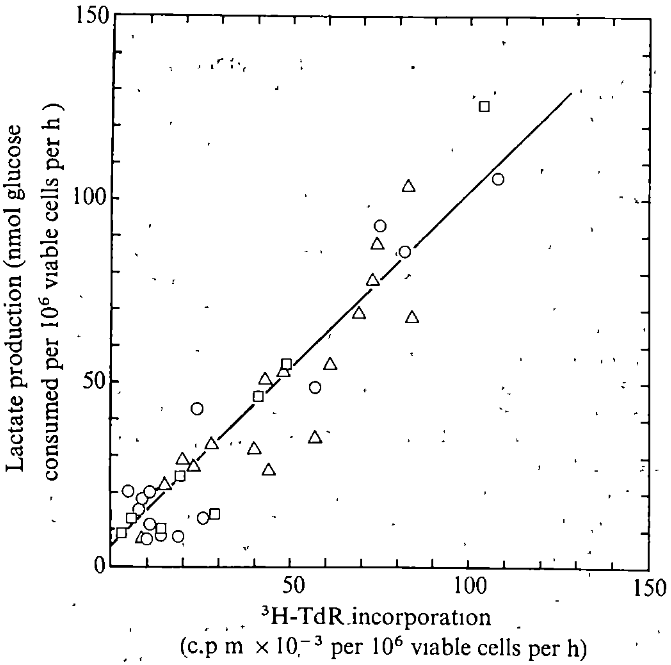

Fig. 2 Lactate production plotted against ^{3}H-TdR incorporation with data obtained from the assays described for Fig 1. The data are from two experiments each with 1.25 × 10^6 cells per ml (□), 2.5 × 10^6 cells per ml (○) and 5 × 10^6 cells per ml (△). All time points from 24–72 h are presented. The line of best fit was obtained through least squares analysis of the data and $r = 0.93$

revealed in Fig. 2. A plot of the rates of ^{3}H-thymidine incorporation against lactate production gave a straight line (r=0.93). But all cells can produce lactate when necessary and before a link between aerobic glycolysis and DNA synthesis can be accepted, explanations based on metabolic artefacts imposed by culture conditions must be considered. The hypothesis has been made that aerobic glycolysis appears as a result of the residence of cells in culture[18]. For the control lymphocytes in Fig. 1*b*, there was no significant increase in aerobic glycolysis during 120 h in culture. Even more convincingly, the high levels of glycolysis found in con A-stimulated cells did not persist with the cessation of DNA synthesis. Therefore, in the absence of proliferation, the metabolism of these cells did not shift towards aerobic glycolysis merely as an adaptation to culture conditions.

The data of Fig. 2 contribute to establishing the relationship between DNA synthesis and aerobic glycolysis in two ways. First, the determinations were done after 24–72 h in culture. Therefore, whether DNA synthesis was just beginning, at a maximum or at an end, a proportional amount of lactate was made. Second, these data are from experiments which used cultures of three different initial cell densities (1.25, 2.5 or 5×10^6 cells per ml). Aerobic glycolysis does not seem to depend on cell density within this range, and the rate remains proportional to DNA synthesis.

Two further groups of experiments were carried out to eliminate other possible explanations for this phenomenon. In the first, the medium and serum were changed every 12 h to ensure the removal of toxic products and the presence of sufficient nutrients so that cellular metabolism would not be influenced. In further experiments culture *p*H was kept constant at either 6.9 with 15 mM PIPES or 7.7 with 10 mM HEPES. No alteration was seen in the relationship of DNA synthesis to lactate production in either experimental group.

These experiments show that aerobic glycolysis considerably increased together with DNA synthesis in con A-stimulated mouse spleen lymphocytes. Furthermore, with the return of these cells to the non-proliferative state, lactate production also fell. For these cells, aerobic glycolysis seems intrinsic to the proliferative response and is not dominated by culture conditions such as cell density, culture age, medium *p*H, exhaustion of nutrients or accumulation of toxic metabolites. Obviously, as other studies have shown, lactate production can be altered to a variable extent by these factors[12,13]. All cells will produce lactate anaerobically and glycolysis will increase when cell density begins to interfere with oxygenation. Similarly, when the glucose concentration is lowered to one tenth of physiological levels and one fourth of the concentration we found optimum for glycolysis by lymphocytes, lactate production by chick embryo fibroblasts will fall[19]. But when we tried to minimise the limitations imposed by the culture conditions, DNA synthesis and aerobic glycolysis seemed to be related. This is not to suggest that they are tightly coupled. Lactate production can and will change independently in varied conditions.

There are other possible explanations for our results. Small lymphocytes, although partially differentiated, are resting cells (G_0 phase) with sparse cytoplasm and a dense nucleus. Stimulation by con A promptly increased RNA and protein synthesis and enlarged the cytoplasm. Micrometric analysis showed that cell volume approximately doubled by the onset of DNA synthesis (our unpublished work). The many cellular changes which follow stimulation by con A presumably also include further differentiation. Con A bound to Sepharose beads has been shown to induce immunoglobulin production, a marker of cell differentiation, as well as cell division in mouse bone marrow lymphocytes[20]. It is therefore possible that the increased aerobic glycolysis we observed is related to cell growth and/or differentiation rather than to DNA synthesis. We believe this is the less likely alternative. According to our results, the temporal correlation of lactate production is much better with DNA synthesis than with RNA and protein synthesis. This suggests that general cell growth is not the source. The process of cellular differentiation, however, does seem to take place together with cell division[21]. Immunoglobulin synthesis, for example, was greatest in late G_1 and early S phase in one study of synchronised lymphocytes[22]. In spite of the apparent dependence of differentiation on cell division, it seems that differentiation is not the stimulus for aerobic glycolysis. Once the differentiated state has been reached, lactate production rarely persists. But the most telling argument seems to be the clearly inverse relationship between aerobic glycolysis and the degree of differentiation of various hepatomas[7]. The final resolution of this question, however, awaits greater understanding of both cell division and differentiation.

We have proposed that the simultaneous decline in DNA synthesis and lactate production is important evidence that these two events are linked. It may reflect, however, a loss of viability of these cells. We believe this is not the case for several reasons. Our results are corrected for cell viability which remains stable from the peak of DNA synthesis (48–60 h) to 120 h. Cellular integrity can also be substantiated by the normal level of lymphocyte respiration observed 96 h after stimulation by con A (our unpublished work). But most important, the cell number continues to increase exponentially up to 96 h—a clear sign of viability.

It is true that the lymphocytes are unresponsive to con A after their proliferative response. We feel that this is to be expected. Stimulation by con A presumably produces the *in vitro* correlate of the final activation and differentiation which occurs *in vivo* in response to an antigenic challenge. It is unlikely that lymphocytes *in vivo* have the capacity for continuous division.

The question of whether or not the cancerous state increases aerobic glycolysis when growth rate is corrected for, has rarely been studied critically. Almost always controls have been normal undividing cells. It has been investigated, however, in chick embryo fibroblasts. In one study, transformation by Rous sarcoma virus did not result in enhanced lactate production[9]. A second group, however, found that if the ratio of aerobic glycolysis to respiration was determined, the ratio was increased by transformation[13]. Consequently, cancerous transformation may lead to a further shift of metabolism towards aerobic glycolysis but the evidence at this time is far from complete.

Our studies have also shown that glucose utilisation rises as expected during active glycolysis and, in addition, glucose transport increases approximately thirtyfold (unpublished work). Catabolism of glucose during this phase results predominantly in the formation of lactate and more than 95% of the label is recovered as either lactate or glucose At present, the reason for these alterations in glucose metabolism during DNA synthesis is unknown. Whatever the explanation proves to be, aerobic glycolysis may be a general characteristic of dividing cells. The Warburg effect, rather than a reflection of defective respiration, may prove to be a normal accompaniment of cell division.

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Further evidence for derepression of H–2 and Ia-like specificities of foreign haplotypes in mouse tumour cell lines

WE reported earlier the appearance of specificities like those of foreign H–2 haplotypes on a mouse sarcoma after vaccinia virus infection[1]. The presence of these foreign transplantation antigens was apparently sufficient to inhibit the growth of the tumour (unpublished data). Parallel work in man has shown qualitative changes in the HLA profile of fibroblasts grown in tissue culture and infected with SV40 virus[2]. The mechanism by which a virus can induce changes in the pattern of cell surface histocompatibility antigens remains obscure.

We recently favoured a derepression hypothesis. According to this hypothesis, allelic differences in histocompatibility antigens are the products of closely linked genes[3] most of which are repressed in normal circumstances. A virus or other agent could interfere with the repression system and cause derepression of certain genes and/or repression of others resulting in the appearance of allogeneic and/or loss of syngeneic specificities. In this report we present further data which are in line with this repression–derepression hypothesis. A spontaneous θ-positive mouse lymphoma (SL2) was found to express in addition to the H–2 specificities of the host's cells, alloantigens (H–2 and Ia) apparently of foreign haplotypes. A chemically induced lymphosarcoma (Gardner) on the other hand was found not to express one of the private H–2 specificities of the host's cells.

Tumour cells were propagated in ascites form in syngeneic recipients (SL2 in DBA/2 (ref. 4) (H–2^d) and Gardner in CBA/H (ref. 5) (H–2^k)). They were taken from the peritoneal cavity, tested in direct complement-dependent microtototoxicity and also with a post-labelling assay measuring uptake of ^{14}C-thymidine. The pattern of reaction of both tumours with a variety of sera of restricted specificity, that is, alloantisera against H–2, non-H–2 (Thy 1.2 and Ly 4.2) and heteroantisera against B cells and mouse Ig is shown in Table 1. SL2 lymphoma cells reacted strongly with all antisera against private and public specificities of the host (haplotype: H–2^d; H–2 specificities: 4,31,3,8,13,28,35). Positive reactions against SL2 were also found with some other anti-H–2 sera which do not react with H–2^d lymphocytes and which are directed against private H–2 specificities of non-H–2^d haplotypes (that is H–2—2,17,19,20,30). Some of the extra reactivities against SL2 were further analysed by specific absorption procedures (Table 2). The cytotoxic effect of anti-H–2.1 and anti-H–2D.32 on SL2 was specifically removed with normal lymphoid cells from H–2.1 and H–2D.32 positive strains and could not be distinguished from the normal H–2 specificities present in the control tumour Gardner expressing H–2.1 and H–2D.32 in accordance with genetic expectation.

The results obtained with Gardner lymphoma show that a tumour may also lose certain H–2 specificities. Antiserum against the private H–2K specificity (H–2K.23) did not react with the lymphoma while it reacted with normal lymphocytes from the host. The absence of K.23 from the tumour cells was confirmed by absorption analysis and by indirect immunofluorescence. Another anti-H–$2K^k$ serum, A.TL anti-A.AL was also negative in the cytotoxicity test. On the other hand, all the sera against public H–2 specificities of the H–2^k haplotype reacted strongly with the tumour and two sera (anti-H–2.16 and anti-H–2.35) gave strong extra reactions.

Ia specificities also seemed to be expressed on one of the Thy 1.2 positive tumours (see the reaction with A.TH anti-A.TL against SL2, Table 1). This was of particular interest in view of the fact that Ia antigens are expressed predominantly on B cells[8] and macrophages[9,10]. Several anti-Ia sera of restricted specificity (kindly provided by Dr Chella David) were titrated against SL2 tumour cells (Fig. 1). Some were strongly and others weakly positive, including a serum which was raised in an H–2^d hybrid mouse (A.TH×B10.Da) anti-A.TL. One serum (anti-Ia 6,7) was found to be negative The cytotoxic anti-tumour activity of anti-Ia sera could be absorbed with spleen cells from mice of foreign H–2 haplotypes (that is B10.BR (H–2^k), B10 A (H–2^a) and B10.A(4R) (H–2^{h4})) but not with spleen cells from H–2 identical mice (B10.D2 (H–2^d)) or from

Fig. 1 Cytotoxicity of anti-H–2D.4 (●), anti-Thy 1.2 (▲) and anti-Ia sera against SL2 tumour cells. A.TH anti-A.TL (○), anti-Ia 1,2,3,7,15,19; (A.CA×B10.HTT) anti-A.TL (△), anti-Ia 2,3,15,19; (A.BY×B10.HTT) anti-A.TL (□), anti-Ia 1,2,15,19; (A.TH×B10.D2) anti-A.TL (*), anti-Ia 1,2,3,19; (129×B10A 4R) anti-B10A 2R (◆), anti-Ia 6,7.

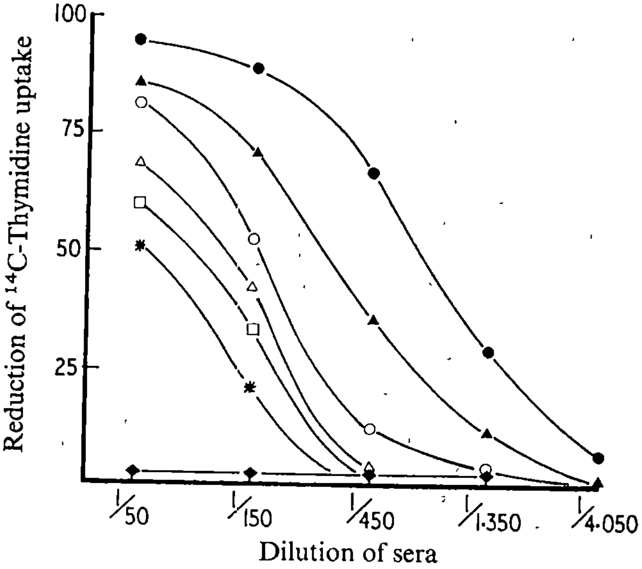

Table 1 Cytotoxic activity of anti-H–2 sera against SL2 leukaemia and Gardner lymphoma

Pretreatment of tumour cells with: Sera	(Haplotype)	C′	SL2(H–2^d) ^{14}C-thymidine uptake c.p m.±s.d.	Reduction (%)	Gardner (H–2^k) ^{14}C-thymidine uptake c.p.m.±s d.	Reduction (%)
—		—	15.792±1.998		12.948± 642	
—		+	12.948± 399		13 539± 924	
Private						
Anti-H–2D 2	(b,g,h,j,)	+	7.119±1.554	45	13 830±1 920	0
Anti-H–2D.4	(a,d,i,t,u,y)	+	1.164± 102	91*	12.861± 954	5
Anti-H–2.9	(f)	+	12.429±1.890	4	13.719±1.329	0
Anti-H–2D.12	(s)	+	13.473± 360	0	13.131± 813	3
Anti-H–2K.15	(j)	+	3.366± 840	74	10.017±1.095	26
Anti-H–2.16	(p)	+	5.826± 378	55	0 945± 144	93
Anti-H–2K.17	(q,y)	+	5.697± 546	56	12.996±1 083	4
Anti-H–2.18	(r)	+	2.328± 222	82	9.882± 732	27
Anti-H–2K.19	(s,t)	+	3.495± 588	73	12.318±1 527	9
Anti-H–2K.20	(u)	+	7.509±1.035	42	13 590±1 143	0
Anti-H–2K.23	(a,h,k,m)	+	14.310±1 617	0	13.653± 888	0†
Anti-H–2D.30	(m,q,v)	+	4.788± 519	63	11.100±1 362	18
Anti-H–2K.31	(d,g,o)	+	0.258± 72	98*	14.307±1.146	0
Anti-H–2D.32	(k,o)	+	3.237± 630	75	0 945± 192	93†
Anti-H–2K.33	(b,i)	+	13.041±1.353	0	11 370± 843	16
Public						
Anti-H–2.1		+	2.847± 306	78	1 488± 297	89†
Anti-H–2.3		+	0.387± 42	97*	0.540± 102	96†
Anti-H–2.5		+	14.418±1.667	0	1 893± 243	86†
Anti-H–2.8		+	0.537± 204	96*	1.281± 72	91†
Anti-H–2.11		+	7 248± 48	44	2 571± 189	81†
Anti-H–2.13		+	0.399± 66	97*	14.349± 657	0
Anti-H–2 25		+	13 284± 369	0	7.581± 735	44†
Anti-H–2.28		+	0.645± 144	95*	13 890± 440	0
Anti-H–2.35		+	0.249± 27	98*	1.083± 219	92
Others						
Normal Mouse Serum		+	12.039± 993	7	12.996±1.083	4
Anti-Thy 1.2		+	0.516± 54	96	1.146± 306	92
Anti-Ly 4.2		+	13 503±1.824	0	13.608±1.527	0
Anti-Ia (A TH anti-A.TL)		+	0.906± 237	93	13 158± 651	3
CBA/H anti-BALB/c		+	0.654± 111	95	9.204± 630	32
BALB/c anti-CBA/H		+	7.770± 942	40	2 463± 354	82
Rabbit anti-MBLA		+	13.446±1.440	0	7.446± 384	45
Rabbit anti-MIg		+	13.173 ±1.899	0	13.818±1 086	0

2 × 10^4 Macrophage-depleted tumour cells (SL2 or Gardner) in 50 μl of culture medium were incubated in round bottom wells of sterile microtitre plates with 1 μl of antiserum for 1 h at 4 °C and then 30 min with 50 μl of prediluted 1/10 rabbit complement at room temperature (C′) For SL2 the rabbit complement was previously absorbed with DBA/2 spleen and thymus cells for 1 h at 0 °C After washing, 10 μl of ^{14}C-thymidine (4 μCi ml^{-1}) were added to the plates for a further 16 h The results are expressed as the percentage reduction in thymidine uptake in test samples compared with control samples with complement and no antibody Similar results were obtained by measuring ^{14}C-uridine uptake. For details of the H–2 alloantisera see NIH catalogue and supplements[6]. A.TH anti-A.TL serum was a gift of Dr Chella David, the other sera were raised and tested for specificity in the London Hospital Medical College.

*Expected positive reaction for an H–2^d cell: private specificities H–2D.4 and H–2K.31, public 3,8,13,28,35.

†Expected positive reactions for an H–2^k cell: private specificities H–2K 23 and H–2.D32, public 1,3,5,8,11,25 (ref 7).

Table 2 Absorption of cytotoxic anti-tumour activity from anti-H–2 sera and anti-Thy 1 2 by normal lymphoid cells and brain tissue carrying the appropriate antigenic determinants

Antisera	Absorbing cells from*	C′	SL2 (H–2^d) ^{14}C-thymidine uptake c.p.m.±s.d.	Reduction (%)	Gardner (H–2^k) ^{14}C-thymidine uptake c p.m.±s d.	Reduction (%)
—	—	—	18.606± 942		12.945± 576	
—	—	+	14.535± 354		14.550±1 134	
Anti-H–2 K 31	—	+	0.579± 117	96	14.601±1.941	5
Anti-H–2 K.31	C57BL.10(31neg)	+	2.355± 54	84	15.540±1 101	0
Anti-H–2 K.31	B10.BR(31neg)	+	1.281± 747	91	14.031± 522	4
Anti-H–2 K.31	B10.D2(31pos)	+	13.113±2.016	11	14.706±2 493	0
Anti-H–2.1	—	+	3.396±1.107	77	3.957± 849	73
Anti-H–2 1	C57BL.10(1neg)	+	2.637± 726	82	5 829± 687	60
Anti-H–2.1	B10.D2(1neg)	+	6.546± 801	55	3.084± 450	79
Anti-H–2.1	B10.BR(1pos)	+	11.751± 882	19	12.108± 576	17
Anti-H–2D.32	—	+	4.233± 525	71	2 178± 423	85
Anti-H–2D.32	B10D2(32neg)	+	7.380±1.284	49	6.813±2 454	54
Anti-H–2D.32	B10 A(32neg)	+	3.054± 369	79	5.781±1.794	60
Anti-H–2D.32	B10.BR(32pos)	+	12.273± 804	17	15 618± 783	0
Anti-Thy 1 2	—	+	1.368± 516	91	1.611± 213	89
Anti-Thy 1.2	CBA/H brain (Thy 1.2 pos)	+	15.390±1.815	0	15.426±1.575	0
Anti-Thy 1.2	AKR brain (Thy 1.2 neg)	+	3.426± 303	76	1 716± 531	88

*H–2 alloantisera prediluted 1/6 or 1/10 and anti-Thy 1 2 were absorbed with 5 × 10^7 spleen cells from B10, B10.BR, B10.D2, B10.A mice or with brain tissue from CBA and AKR mice respectively for 1 h at 4 °C and tested back against SL2 (H–2^d) and the control tumour Gardner (H–2^k) The results are expressed as % of reduction in ^{14}C-thymidine uptake in test samples as compared with control samples with complement (C′) and no antibody.

Table 3 Absorption of cytotoxic anti-tumour activity against SL2 leukaemia from anti-Ia sera by normal lymphoid cells carrying the appropriate Ia determinants

Antisera	Absorbing cells from*	Potential anti-Ia activity	C′	^{14}C-thymidine uptake c.p.m.±s.d.	Reduction (%)
—	—	—	—	10.158± 333	
—	—	—	+	8.505± 312	
(A.CA × B10.HTT) anti-A.TL	—	2, 3, 15, 19	+	2 466± 126	71
(A CA × B10.HTT) anti-A.TL	B10 HTT (H–2^{t3})	2, 3, 15, 19	+	3.168± 384	63
(A.CA × B10.HTT) anti-A.TL	C57BL.10(H–2^{b})	2, 15, 19	+	3.810± 132	55
(A.CA × B10.HTT) anti-A.TL	B10.BR (H–2^{k})	—	+	6 408± 675	25
(A.CA × B10.HTT) anti-A.TL	B10.D2 (H–2^{d})	2, 3, 19	+	3.996± 843	53
A.TH anti-A.TL	—	1,2, 3,7, 15, 19	+	0.835± 252	90
A.TH anti-A.TL	A.TH (H–2^{t2})	1,2, 3,7, 15, 19	+	2.410± 513	72
A.TH anti-A.TL	B10.A(4R) (H–2^{h4})	7, 15, 19	+	6.578± 704	22
A.TH anti-A.TL	B10.A (H–2^{a})	—	+	6.431± 750	25

*60 µl of 1/6 or 1/10 diluted antiserum was absorbed with 5×10^7 spleen cells from B10, B10 D2, B10.BR, B10.A, B10.A(4R), B10.HTT or A.TH mice for 1 h at 4 °C and tested back against SL2 cells in presence of complement. Tumour cells were postlabelled with ^{14}C-thymidine overnight. Results are expressed as % or reduction in ^{14}C-thymidine uptake compared with complement alone (C′). Ia specificities in H–2^{d} haplotypes 6,7,8,11,15,16 (ref. 11).

serum donor strains (B10.HTT, A.TH) (Table 3). All data taken together suggest that the SL2 lymphoma expresses Ia specificities of foreign haplotypes (most likely Ia 1,2,3). Of particular biological importance now will be to establish whether the H-2- and Ia-like specificities of foreign haplotypes on SL2 are the tumour specific transplantation antigens which have already been isolated and partially purified from these tumour cells[12].

A theoretical possibility is that a virus on the surface of a cell and in close proximity to a histocompatibility antigen could produce a new antigen pattern which resembles a foreign transplantation antigen. If this were the explanation we should expect anti virus activity to be removed by absorption with normal lymphoid cells and the normal H–2 configuration to be altered[13,14]. We could find no alteration of the normal H–2^{d} profile, nor was it possible to remove anti-virus activity with normal lymphoid cells. The same NIH reference sera which caused anomalous anti-tumour reactions in Nowinski's and Klein's studies[15] (D-4 and D-31) reacted normally with all our tumour cells (F.G., V.S. and H.F., unpublished). Furthermore, it is unlikely that extra reactions are due to "cross-reacting antigens" in damaged cells because our assay measures only metabolic activity in healthy cells.

From our specific absorption data we favour the above-mentioned derepression hypothesis, although we cannot formally exclude the possibility of activated endogenous virus directly creating new H-2-like determinants on the tumour cell surface. A derepression mechanism would imply that every member of a species carries a full set of structural genes for all the different 'alleles' of a particular system. Since gene repression is much more extensive than gene expression, it would be surprising if the species genes were not widely distributed. Several recent findings which could be explained by derepression of structural genes, normally silent, are: (1) the appearance of an unexpected human allotype in lymphoid tumour lines transferred into hamsters[16], (2) the transient and unpredictable expression of IgG^{a} allotype in an IgG^{b} mouse[17], (3) expression of allotypes of the "wrong kind" in rabbits after immunisation with *Micrococcus lysodeikticus*[18], (4) the appearance and disappearance of HLA antigens in cultured lymphoblastoid cell lines[19,20] and (5) variations of the expression of HLA antigens on human fibroblasts *in vitro*[21].

If there are depressed H-2-like antigens one would expect to find also examples of repression. This could be an explanation for the loss of the K^{k} private specificity of Gardner lymphoma Experiments are under way to find out whether the cells could be activated (derepressed) to produce the missing H-2K^{k} antigen.

Whatever the precise mechanism of the production of the observed extra specificities, the fact that they were serologically indistinguishable from major histocompatibility complex (H–2 and Ia) specificities, opens a new conceptual way of looking at the question of surveillance of tumours and transplants.

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Release and synthesis of prolactin by rat pituitary cell strains are regulated independently by thyrotropin-releasing hormone

THYROTROPIN-releasing hormone (TRH) is the tripeptide pyroglutamyl-histidyl-proline-NH$_2$, which causes the release of prolactin and thyrotropin from the anterior pituitary gland in

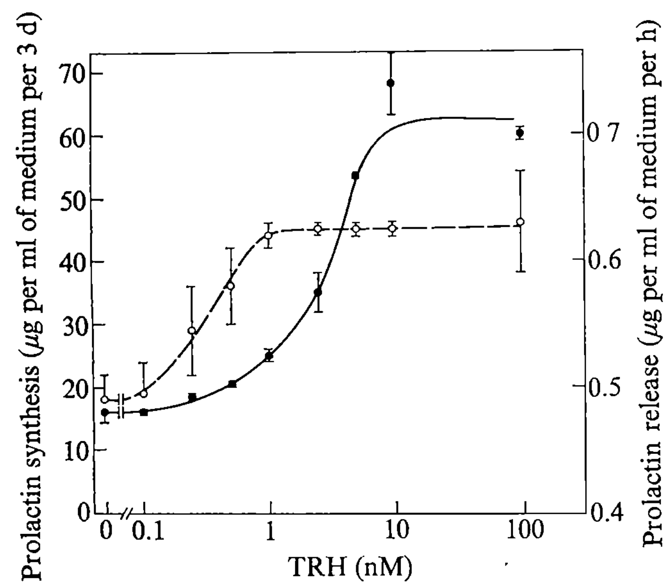


Fig. 1 Effects of increasing concentrations of TRH on prolactin synthesis (●) and release (○) GH cells were plated and grown to about 0.5 mg protein per dish in Ham's F10 medium (Gibco) supplemented with 15% horse serum and 2.5% foetal calf serum[8]. All the experiments described here were then performed in serum-free medium as follows. Three to four days after plating, the medium was removed, the plates were rinsed with 1 ml of Newman and Tytell serum-free medium (Gibco) and then 2.5 ml of this medium were added to each 60-mm culture dish. Solutions of TRH or analogues were added in 0.1 ml of sterile 0.15 M NaCl to give the indicated final concentrations of TRH At the end of 1 h, medium was collected for determination of prolactin release; fresh medium was added and prolactin synthesis was determined 3 d later. Prolactin was measured by microcomplement fixation[6]. The cells do not store much prolactin; therefore measurements made at 3 d reflect almost entirely (99%) hormone synthesis[7]. In the dose-response curves here and in Fig. 2, each point represents the mean of determinations from duplicate dishes; vertical bars give the ranges; ○, release; ●, synthesis.

man and other animals[1–3]. Although the isolation of TRH was based on its ability to release thyrotropin, injection of TRH usually releases both prolactin and thyrotropin[4,5]. In spite of this knowledge, the physiological role of TRH in the control of either thyrotropin or prolactin secretion is not clear. We report here that the release and synthesis of prolactin are controlled independently by TRH in rat GH cells.

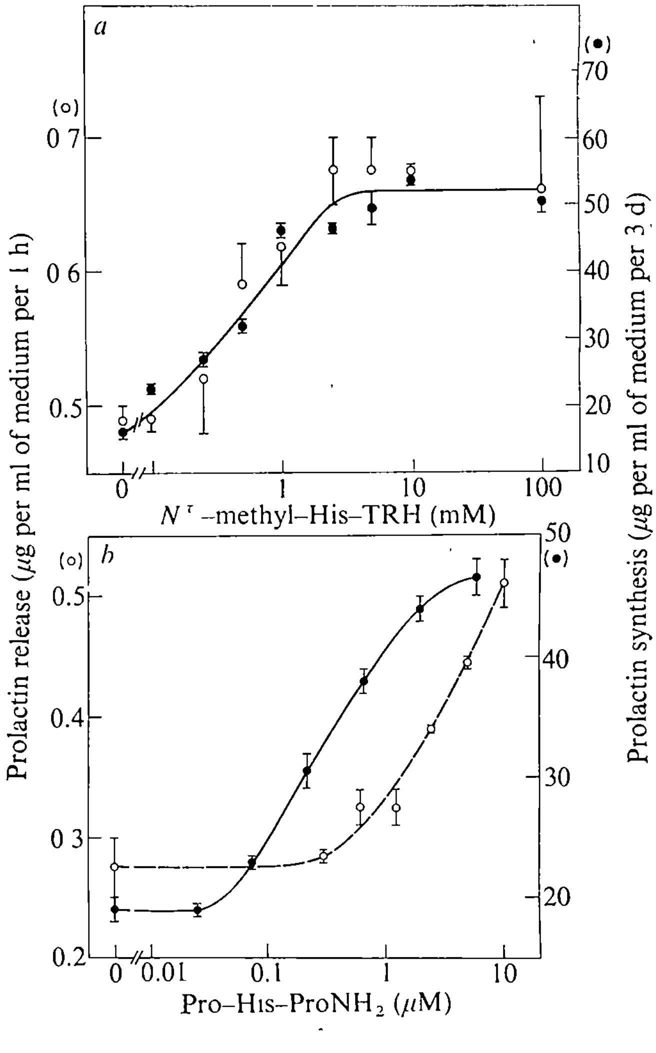
GH cells are clonal strains of rat pituitary cells in culture which synthesise and secrete prolactin and growth hormone, but not thyrotropin or other anterior pituitary hormones[6]. TRH stimulates the release of prolactin from GH cells within minutes; later, 3–4 h after addition of TRH to the medium, the synthesis of prolactin begins to increase and reaches a maximum 24–48 h later[7].

Dose-response curves for the TRH-induced increases in prolactin release and synthesis are shown in Fig. 1. Half-maximal stimulation of prolactin release occurred at 0.3 nM TRH; the half-maximal increase in synthesis occurred at 3 nM. Three possible explanations for this difference are as follows. (1) TRH is not stable in the medium and therefore the concentration of peptide is decreasing during a 3-d synthesis experiment. (2) Fewer TRH receptors need to be occupied to stimulate prolactin release than to stimulate its synthesis. The binding of TRH to GH cells has been measured; there is half-maximal binding at 11 nM (ref. 9). Because half-maximal stimulation of prolactin synthesis occurs at 3 nM, it seems that not all receptors need to be occupied to stimulate synthesis, and occupancy of even fewer may be required to cause release. (3) The hormone-receptor interaction that mediates prolactin release is different from that which mediates synthesis. We have investigated these possibilities.

TRH that is bound to GH cells is degraded[10]; however, after 3 d this amounts to less than 1% of the TRH present in the medium, a finding which cannot account for the tenfold difference between TRH concentrations required for release and synthesis. Although TRH is degraded in whole serum[11,12] we have shown that the peptide is stable in the conditions of these experiments in serum-free medium. The serological activity of TRH that had been incubated with GH cells for 3 d was the same as that of unincubated TRH with an antiserum to TRH which does not react with pGlu–His–Pro, one of the major degradation products of TRH (unpublished results). In addition, the biological activity of TRH is not lost after incubation with the cells. TRH (300 nM) was incubated with GH cells for 3 d and then aliquots of this medium were tested for prolactin synthesis-stimulating activity by incubation with fresh cells for a further 3 d. Half-maximal stimulation by TRH which had been incubated with GH cells occurred at 3 7 and 1.6 nM in two separate experiments, which is in agreement with the value of 3 nM (Fig. 1) for unincubated TRH.

If the second possibility were true, that is that fewer receptors need to be occupied to stimulate prolactin synthesis than to stimulate release, then only one receptor would be involved, and any active analogue should cause release at lower concentrations than synthesis. Data in Fig. 2 reveal that this is not true. An analogue of TRH (Fig. 2*a*) with a methyl group on the third

Fig. 2 Effects of increasing concentrations of two analogues of TRH on prolactin release and synthesis. *a*, Effects of pyroglutamyl-N^{τ}-methyl-histidyl-proline-NH_2, *b*, prolyl-histidyl-proline-NH_2. Other experiments showed that a maximum increase in synthesis had been reached with the highest concentration of prolyl-histidyl-proline-NH_2 that is shown Experimental procedures were as described in the legend to Fig. 1. ●, Synthesis; ○, release.



nitrogen of the imidazole ring of the histidine (*N*τ-methyl-histidyl-TRH) caused half-maximal stimulation of release and synthesis at the same concentration, about 0.5 nM. Another analogue (Fig. 2*b*) with a proline residue substituted for the pyroglutamyl ring was more active in stimulating synthesis than release, so that half-maximal stimulation of synthesis (0.25 μM) was reached before there was any detectable increase in release. This finding demonstrates that increased release of prolactin is not necessary to trigger increased synthesis, and that synthesis can be stimulated independently.

Table 1 summarises the results for the analogues of TRH that we have tested. Variations shown in the pyroglutamyl and histidyl residues all influenced release more than they did synthesis, resulting in ratios of synthesis to release of less than 10 compared with a ratio of 10 for TRH. Modifications in the C-terminal proline amide residue in most cases seemed to affect release and synthesis to similar extents, for with these analogues the ratios of synthesis to release remained about 10 or greater. One analogue, pGlu–His–ProNH$(CH_2)_6NH_2$, did not fall into this category. It may be that this peptide is not stable and is metabolised into a more active compound; studies are in progress to test the reason for this discrepancy.

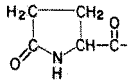
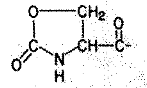
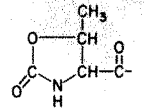
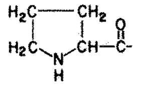
These data are the first evidence of which we are aware that hormone release and synthesis can be influenced separately by a single regulatory factor.

Since prolactin release and synthesis can be affected differently by analogues of TRH, the pathways through which these two processes are mediated must be separate. The experiments performed so far have involved agonists of TRH; the different responses to different agonists indicate that there are two separate interactions, but they do not prove there are two physically distinct sites. For example, TRH might bind to one site, activating adenylate cyclase to enhance release, while causing aggregation of receptors to stimulate synthesis: modifications in the structure of TRH could influence one process and not the other. An antagonist of TRH that would be capable of interfering with one process and not the other would demonstrate that the two sites are physically separate.

The TRH receptor in GH cells has been characterised and only one class of high-affinity sites has been detected; there is half-maximal saturation of these sites at 11 nM compared with 2–3 nM for the half-maximal increase in prolactin synthesis[9]. The *N*τ-methyl-histidyl analogue competes with ^{3}H-TRH for binding more effectively than does TRH itself[13]. The affinity of the receptor and the specificity of the methyl derivative indicate that the characterised receptor has the properties that would be expected if it were involved in synthesis and not release. If a separate receptor for release exists, it must therefore be present in smaller numbers than the synthesis receptor.

Some analogues of TRH used in this study have also been tested for their effects on the release of thyrotropin in intact animals and from isolated pituitary glands. When data from this report on prolactin release are compared with data on thyrotropin release from normal pituitary glands, the relative activities of most analogues compared with TRH are very similar[14,15], this is also true in one report in which release of both prolactin and thyrotropin was measured, using several TRH analogues, in rats[3]. These findings suggest that the

Table 1 Half-maximal concentrations of TRH and analogues of TRH required for stimulation of prolactin release and synthesis

Peptide	Release [peptide] for half maximum (nM)	Synthesis [peptide] for half maximum (nM)	[Peptide] for synthesis / [Peptide] for release
X–His–ProNH$_2$			
TRH 	0.3	3	10
	1	3.5	3.5
	5	3.5	0.7
	1,250	250	0.2
pGlu–X–ProNH$_2$			
pGlu–*N*τ–MeHis–ProNH$_2$	0.5	0.5	1
pGlu–*d*His–ProNH$_2$	25	100	4
pGlu–Lys–ProNH$_2$	>2,000	>2,000	—
pGlu–Arg–ProNH$_2$	>2,000	>2,000	—
pGlu–His–X			
pGlu–His–Pro	200	4,000	20
pGlu–His–ProNHCH$_3$	4	50	12
pGlu–His–*d*ProNH$_2$	300	3,000	10
pGlu–His–isoaminobutyric acid	300	3,000	10
pGlu–His–ProNH$(CH_2)_6NH_2$	300	20	0.07
pGlu–His–ProN$(CH_3)_2$	>1,000	>1,000	—

Effects of each analogue on prolactin release and synthesis were measured in complete dose–response experiments such as those shown in Figs 1 and 2. These values are the result of at least two dose–response curves; the half-maximal values in separate experiments did not differ more than twofold.

receptor mediating thyrotropin release is similar to the receptor mediating prolactin release. But one example in which the response to an analogue is not similar for prolactin and thyrotropin is the *N*τ-methyl-histidyl analogue which is eight times more active than TRH in stimulating thyrotropin release in intact animals[14], but is not more active than TRH in causing prolactin release from GH cells. The effect of the *N*τ-methyl-histidyl analogue on prolactin release in intact animals has not yet been reported. If GH cells are a valid model system for the normal pituitary gland, this derivative should not be more active than TRH in releasing prolactin in the normal pituitary gland. When further comparisons are made, other differences between prolactin release and TSH release may be discovered.

TRH has been reported to affect the central nervous system, for example, enhancing behavioural excitation produced by a combination of tranylcypromine and L-tryptophan[16], and causing rapid hypothermia when injected intraventricularly in the cat[17]. TRH inhibited the firing of some neurones of the hypothalamus when applied microiontophoretically[18], and it has been suggested as a neurotransmitter[19]. It therefore may not be surprising that TRH, like other established neurotransmitters, has more than one receptor.

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Immunofluorescence labelling of gonadotropin receptors in enzyme-dispersed interstitial cells

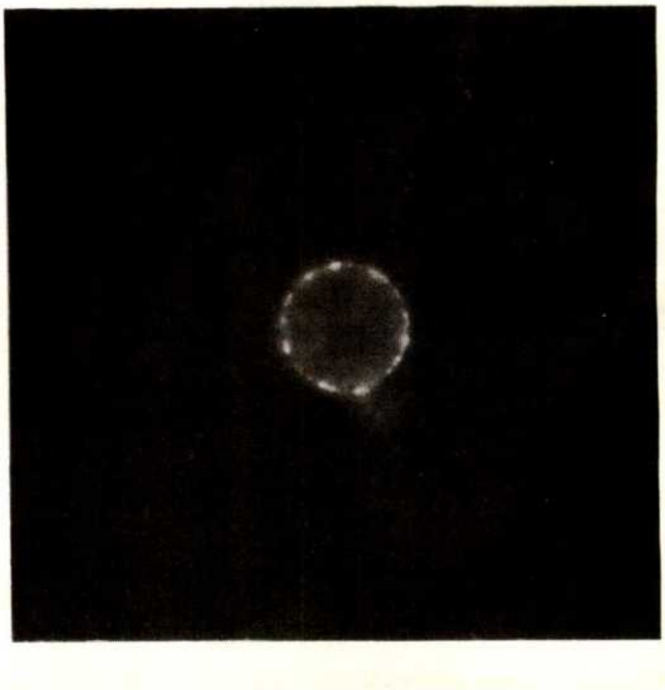
PEPTIDE hormone receptors in target tissues have been extensively analysed *in vitro* by binding studies with radioactive hormone derivatives. Although these methods have been of great value for characterisation of the binding sites for peptide hormones, there is also need for methods to examine the localisation and distribution of hormone receptors in isolated target cells. For this purpose, we have used an immunofluorescence technique to label the luteinising hormone (LH) receptors of the interstitial cells of the rat testis. This method has demonstrated that LH receptors are distributed as patches on the cell surface, and are rapidly lost from about 50% of the labelled cells after hypophysectomy. Also, occupancy of receptors by endogenous LH was found to persist for several days in a significant proportion of the interstitial cells.

Specific receptor sites for LH were originally identified in the interstitial cells of the testis by autoradiographic and immunohistological procedures[1,2]. LH receptors have also been characterised *in vitro* by binding studies with radioiodinated LH or human chorionic gonadotropin (hCG) in testis homogenates and dispersed interstitial cells[3–6]. The gonadotropin receptors of the rat testis have been shown to be associated with the plasma membrane of the interstitial cell during sucrose density gradient centrifugation and hormone binding studies[7]. In addition, about 50% of enzyme-dispersed testicular interstitial cells have been shown to possess steroidogenic enzyme activity characteristic of Leydig cells, the LH-responsive androgen-secreting cells of the interstitium[6]. The localisation and distribution of LH receptors in isolated interstitial cells has now been examined by an indirect immunofluorescent method using high titre rabbit antiserum to hCG and fluorescein-conjugated goat anti-serum to rabbit 7S globulin, after exposure of the cells to a saturating concentration of hCG.

Interstitial cell suspensions were prepared from decapsulated testes of mature Sprague–Dawley rats by collagenase dispersion as described previously[6–8]; all incubation and washing steps being carried out in medium 199 containing 0.1% BSA. To saturate the LH receptors with gonadotropin, 0.5-ml aliquots of the cell suspension containing 5×10^6–10×10^6 cells ml^{-1} were incubated in plastic centrifuge tubes with 100 IU hCG (Pregnyl, Organon) at 34 °C for 30 min. Cells were then washed twice with 40 ml medium, sedimented at 500*g* for 10 min, and resuspended in 0.6 ml medium before incubation at 34 °C for 30 min with rabbit anti-hCG serum at a final dilution of 1: 60. The titre of the antiserum, in terms of the dilution required to bind 50% of a tracer quantity (50 pg) of ^{125}I-labelled hCG (100 μCi $\mu\mathrm{g}^{-1}$), was approximately $1:10^6$. The cells were then washed four times with 40 ml medium and resuspended in 0.3 ml medium, containing 5 μl fluorescein-conjugated goat antiserum to rabbit 7S γ-globulin (Hyland, California; fluorescein–protein molar ratio, 3.6; 15.0 mg total protein ml^{-1}). After incubation for 30 min at 4 °C, the cells were washed twice with 40 ml medium and examined under a Leitz epi-illuminated fluorescence microscope.

In preparations obtained from intact rats, 50% of the

Fig. 1 Immunofluorescent labelling of collagenase-dispersed interstitial cells from intact rat testis, showing bright speckling around the circumference of the cell (× 1,350).



interstitial cells exhibited marked fluorescence. This proportion corresponds to that previously observed to possess 3β hydroxysteroid dehydrogenase activity on histochemical analysis of enzyme-dispersed interstitial cells from the rat testis[6], and represents the Leydig cell population of the testis interstitium cells When such cells were examined by focusing on the equatorial plane, a brightly speckled band of fluorescent spots was visible around the circumference of the cell (Fig. 1) Adjustment of the depth of the visual field revealed discrete fluorescent areas over the entire cell surface. In most cells, aggregates of fluorescent spots could also be seen, but no polarisation of the fluorescent patches was observed.

At least 100 cells were counted from each cell preparation by two independent observers Of the control cells, 10±2% (s.e.) exhibited positive fluorescence when anti-hCG serum was replaced by normal rabbit serum or antiserum to angiotensin I. Such control preparations were evaluated with each experimental group, and the number of non-specifically labelled cells was subtracted from the total positive cell count. As shown in Fig. 2, approximately 40% of the interstitial cells from intact animals exhibited specific positive fluorescence. In these animals, significant fluorescent labelling could also be demonstrated in a high proportion (about 30%) of cells which had not been saturated with hCG before immunofluorescent labelling. This suggested that hCG antibodies could be taken up by endogenous LH bound to receptor sites on the cell surface. The ability of the hCG antiserum to react with rat LH was confirmed by the demonstration of direct binding of ^{125}I-labelled rat LH, and by measurement of the binding affinities for ^{125}I-labelled hCG (10^{11} mol^{-1}) and rat LH (about 10^{8} mol^{-1}). Although the affinity of the antiserum for rat LH was considerably lower than the affinity for hCG, the combination of moderate affinity for rat LH and extremely high titre was adequate to account for the observed binding of antibody to endogenous LH present on the cell surface.

Immunofluorescence labelling of LH receptors was also carried out on interstitial cells obtained from mature animals at various intervals after hypophysectomy. Such cell preparations showed a significant decrease in fluorescent labelling carried out with or without saturation by hCG during the first incubation. The percentage of fluorescent cells in hCG-treated preparations decreased by 50% on day 6 after hypophysectomy, and remained constant at this level for up to 18 d. The initial fall in receptor sites, and the relatively constant level thereafter, are consistent with previous observations which demonstrated that hypophysectomy was followed by a decline in binding capacity within the first 3 d of operation, and a subsequent plateau at 30–50% of the control level[7,9].

Our observations suggest that hypophysectomy is followed by a rapid loss of receptor sites in about 50% of the Leydig cells in the testis interstitial cell fraction, rather than by a more generalised decrease in the number of receptor sites per cell. In the latter case, a moderate decrease in immunofluorescence would be observed in all cells, rather than the loss of a fraction of the labelled cell population which occurred after hypophysectomy. These findings suggest that a pituitary factor, possibly LH itself or prolactin, is necessary for maintenance of the LH receptors in a proportion of the Leydig cell population. In other Leydig cells, gonadotropin receptor sites are maintained for more prolonged periods after hypophysectomy, and represent a more stable population which is relatively independent of pituitary control.

In cell preparations not pretreated with hCG, the initially high level of fluorescence is attributable to occupancy of receptors by endogenous LH Such fluorescence decreased in a serial fashion after hypophysectomy, but did not become undetectable until almost 2 weeks had elapsed. Prolonged binding of endogenous gonadotropin to receptor sites is an unexpected finding in view of the general belief that hormone and drug actions are terminated when receptor sites are vacated by the regulatory ligand. Previous *in vitro* studies with labelled gonadotropins indicated that dissociation of hCG from testicular receptors is probably an extremely slow process[4,10,11]. Our demonstration of prolonged occupancy by endogenous gonadotropins suggests that dissociation of the hormone–receptor complex also proceeds slowly *in vivo*, and that occupied receptors may be rendered inaccessible to further hormone stimulation for a prolonged period. A final speculation to be drawn from these studies is that the receptors for gonadotropin may function only once, and that occupied receptors could be inactivated or degraded, rather than reutilised during future hormonal stimulation. The use of immunofluorescent methods for detection of peptide hormone receptors in isolated target cells has considerable potential for investigation of the turnover, regulation and organisation of receptors within the cell membrane.

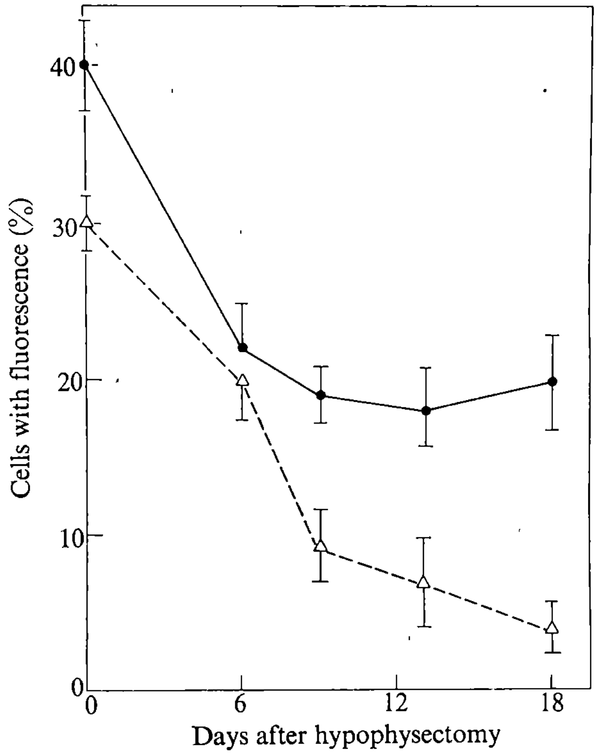


Fig. 2 Quantitative changes in immunofluorescence labelling of collagenase-dispersed interstitial cell preparations from mature male rats during the 3 weeks after hypophysectomy. Immunofluorescence studies were carried out on cells preincubated with (●) or without (△) 100 IU hCG. Each point represents the mean±s.e. of six determinations.

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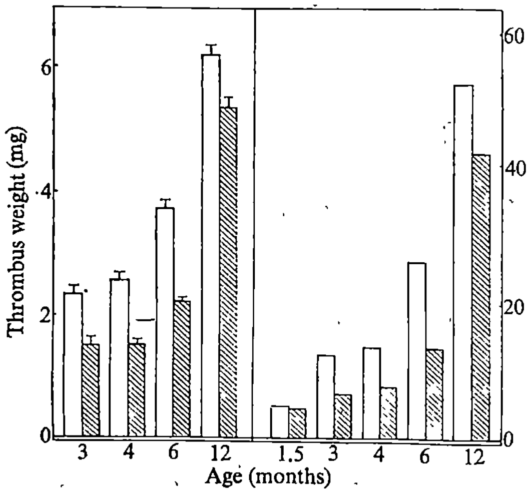
Effect of testosterone, sex and age on experimentally induced arterial thrombosis

The widespread use of large doses of oestrogenic steroids as contraceptive agents or for maintenance therapy in postmenopausal women has led to damaging effects on the cardiovascular system. Most of the reports relate to the increased thromboembolic phenomena which have been observed in both women and men treated with female hormones[1,2]. In sharp contrast there are no data on the effect of androgenic steroids on the development of arterial thrombosis, in spite of the fact that the incidence of ischaemic heart disease in human males is much greater than in the pre-menopausal females[3].

Hornstra and Vendelmans-Starrenburg[4] have described an ingenious method for the induction of experimental arterial occlusive thrombi in rat. The method results in 100% incidence of thrombosis and yields highly reproducible results. Histological analysis of the thrombi showed that their structure and composition is comparable to arterial thrombi found in humans. We used this technique to test the effect of testosterone or oestradiol on thrombus formation in rats of different age groups since the incidence of primary thrombosis (ischaemic heart disease and cerebrovascular accidents) is known to increase with age in both males and females[3]. A polyethylene loop-shaped cannula was inserted in the abdominal aorta of 229 Wistar rats of both sexes aged 1.5, 3, 4, 6 or 12 months. The mortality rate, dry thrombus weight and obstruction time were used as criteria for the degree of the development of occlusive arterial thrombosis.

This phenomenon was found to be significantly more manifested in male than in female rats which were subjected to the same endothelial trauma (Figs 1 and 2). The mortality rate of animals 3, 4, or 6 months of age was approximately twice as high in male rats as in females ($P < 0.01$). This rate increased with the age of the animal (Fig. 1). Twelve-month-old male rats had the highest mortality (52.2%). Female rats at the same age also had a high mortality rate (42.1%), but it was not significantly different from that of the males ($P > 0.05$). The only exception to this observation was in 45-d-old rats, where the mortality rate of these younger animals was lower than in the older rats; no significant difference in mortality was detected between males (4.6%) and females (4.3%) (Fig. 1).

Fig. 1 Thrombus weight (± s.e.m.) and mortality rate of normal male (open columns) and female rats (hatched) at different ages.



Thrombus size was determined in animals killed 5 d after the aorta loops had been inserted. The excised aorta was perfused with saline, opened longitudinally and the thrombus removed. After drying over silica gel *in vacuo* for 24 h, the thrombus material was weighed. No measurable thrombus formation was observed in young rats of either sex at the age of 45 d. Statistically significant differences were found in the size of the thrombi between male and female rats at the age of 3, 4 and 6 months ($P < 0.01$) (Fig. 1). In 6-month-old animals this difference was most pronounced. After this age both males and females became increasingly responsive to the damage inflicted, and the differences between the males and females were no longer significant ($P > 0.05$). We observed no relationship between the thrombus and the body weight of the animals in the same group.

The obstruction time was determined by observing the colour of the blood which flows through the part of the cannula that projects out of the body. The obstruction time decreased progressively in males as well as in females at 3, 4, 6 and 12 months (Fig. 2). There were statistically significant differences ($P > 0.01$) in the obstruction time of male and female rats at the age of 3, 4 and 6 months. Again, sex differences were no longer apparent at 12 months (Fig. 2). During the experimental period (5 d) no obstruction of the loops occurred in animals of both sexes at the age of 45 d.

Species differences may be significant in the incidence of thrombus formation in the two sexes. In consequence, arterial thrombosis in 27 male and female New Zealand rabbits aged 6 months was studied using the method of Maekawa *et al.*[5]. Thrombus formation in the femoral artery was observed in 90.9% of male rabbits, whereas the occurrence of thrombus was established in 56.2% of females ($P < 0.01$).

Testosterone and oestradiol effects were studied in another series of experiments, using 194 Wistar male and female rats, aged 3 months. Depo-testosterone cypionate (Upjohn; 10 mg kg^{-1}) was injected subcutaneously twice a week for four consecutive weeks. Depo-oestradiol cypionate (Upjohn) was administered subcutaneously at a dose of 100 $\mu g\ kg^{-1}$ with the same schedule. Control animals of both sexes received cottonseed oil (0.1 ml subcutaneously). Occlusive arterial thrombosis was induced and the mortality rate, dry thrombus weight and obstruction time were determined. The mortality rate in the

Table 1 Effect of testosterone, oestradiol and Flutamide on mortality rate of rats with occlusive arterial thrombosis

Group	Sex	N	Mortality rate (%)	Significance
Control	Male	28	14.3	
	Female	27	7.4	
Testosterone	Male	48	62.5	$P < 0.001$*
	Female	35	34.3	$P < 0.02$*
Oestradiol	Male	24	8.3	$P > 0.05$*
	Female	32	6.3	$P > 0.05$*
Flutamide + testosterone	Male	30	33.3	$P < 0.02$†
	Female	42	16.7	$P < 0.05$†

Depo-testosterone cypionate (10 mg per kg body weight) was injected subcutaneously twice weekly for 4 consecutive weeks. Depo-oestradiol cypionate (100 µg per kg body weight) was administered subcutaneously on the same schedule as testosterone. Flutamide (50 mg per kg body weight) was injected daily subcutaneously for 2 weeks. Control animals received cottonseed oil (0.1 ml subcutaneously).

*Compared with control rats of the corresponding sex.

†Compared with testosterone-treated rats of the corresponding sex.

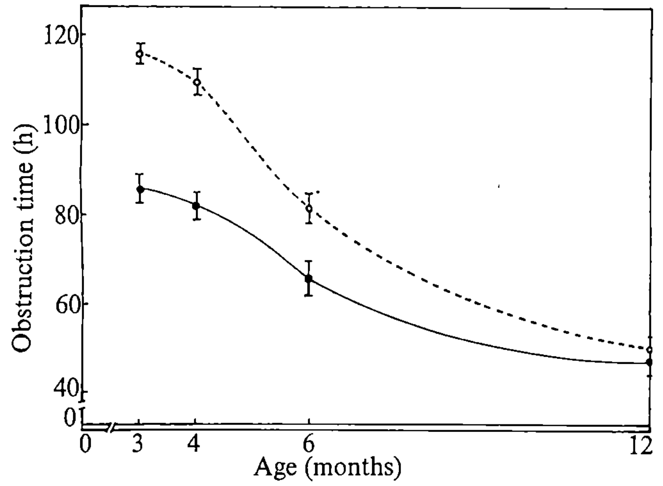


Fig. 2 Obstruction time (± s.e.m.) in rats of different age. ●, Males; ○, females.

control groups, injected with cottonseed oil, was comparable with those of 4-month-old untreated rats (Fig. 1 and Table 1). Testosterone treatment increased the mortality rate more than fourfold in male as well as in female rats (Table 1). Results from oestradiol treatment showed a tendency toward a decrease in the mortality in both sexes; however, this effect was not statistically significant ($P>0.05$).

The values for thrombus weight and obstruction time of the 4-month-old rats from our earlier experiments were comparable with those of the controls injected with cottonseed oil (Figs 1, 2 and 3). Thrombus weight in male rats treated with testosterone was markedly increased ($P<0.001$) reaching an overall maximum of 12.10 ± 0.45 mg. Testosterone treatment of the female animals increased thrombus weight about threefold ($P<0.01$) (Fig. 3). Oestradiol decreased significantly ($P<0.05$) the thrombus weight in male rats only (Fig. 3), but had no significant effect in the females.

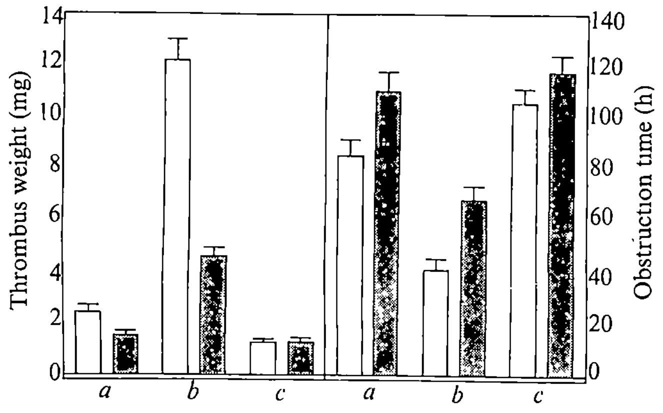


Fig. 3 Thrombus weight and obstruction time of testosterone- (*b*) and oestradiol-treated rats (*c*), compared with control (*a*) male (open columns) and female rats (solid). ± s.e.m. indicated.

Testosterone treatment shortened the obstruction time in both male and female rats ($P<0.01$). Oestradiol increased significantly ($P<0.05$) the obstruction time of male rats only, but did not show an appreciable effect in females (Fig. 3).

Flutamide (α-α-α-tri-fluoro-2-methyl-4-nitro-*m*-propionotoluidide; Schering) a non-steroidal anti-androgen was tested together with testosterone in animals of both sexes. Occlusive arterial thrombosis was induced in male and female rats treated with a combination of Depo-testosterone cypionate 10 mg per kg body weight subcutaneously twice a week, and Flutamide 50 mg per kg body weight subcutaneously daily for 2 weeks. The anti-androgen significantly decreased the mortality rate of both male and female rats when treated with testosterone alone (Table 1).

These data indicate that there is a significant sex difference in thrombus size, obstruction time and mortality rate in rats with occlusive arterial thrombosis, males having approximately twice the thrombus size and death rate compared with females; thrombus weight and mortality rate increases with age; pronounced sex differences also exist in the susceptibility to arterial thrombi formation in rabbits; sex differences in thrombus size, obstruction time and mortality rate in rats are exacerbated by injection of testosterone but not by oestradiol; and the androgen effect in the development of arterial thrombosis seems to be specific since the anti-androgen, Flutamide, significantly reduced the mortality rate in rats treated with testosterone.

These data are consistent with the observation of Amos *et al.*[6] that the amount of dog serum required to produce venous thrombosis in rats is 10–20 times higher for female than for male recipients, and that testosterone treatment of female rats reduced the required dose of serum to that of the male.

The enhanced arterial thrombi formation in male rats is also consistent with the much greater observed[7] ability of platelets to aggregate in male than in female rats and guinea pigs. Similarly, the exacerbating effect of testosterone on thrombus formation is in accordance with previous data showing that testosterone pretreatment enhances platelet aggregability in both sexes[8], which may be mediated through the prostaglandin system[9].

Our results provide for the first time significant experimental evidence for an association between sex and age and the development of arterial thrombosis. These data may be relevant to epidemiological studies[3] showing that arterial thrombosis and related ischaemic heart disease are less common in women during their reproductive period. The observed higher mortality rate in 12-month-old female rats seems to be comparable with the increased vascular occlusion in women after the menopause[10].

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Cholecystokinin both stimulates and inhibits human food intake

THE hypothesis that gastrointestinal hormone(s) released into the blood during normal digestion should inhibit further food intake is attractive. It has been reported[1–4] that injection of cholecystokinin (CCK) inhibited eating in rats and rhesus monkeys. We now report that intravenous infusion of CCK in a dose which produces physiological effects ascribed to CCK during normal digestion stimulated, rather than inhibited, eating in man. In contrast, rapid intravenous injection of CCK inhibited human food intake.

Healthy, non-obese subjects (six men, four women, mean age 33 yr) gave informed consent to participate. A minor modification of the method of Jordan *et al.*[5] for studying food intake was used. After an overnight fast, the subject ate refrigerated Ensure (Ross, Columbus, Ohio; a liquid mixture of fat, carbohydrate, protein, minerals, and vitamins, containing 1.06 kcalorie ml^{-1}) for breakfast; it was suspended in a graduated cylinder at the level of the subject's head, out of sight. It was transmitted through a No. 14 French rubber tube to the subject who ate by opening a pinchcock valve and gently sucking on the tube. Subjects were instructed to eat until their appetite was satisfied, to complete their meal within 20 min, and to report any untoward sensations. The volume of meal ingested was recorded at 1-min intervals.

Immediately before eating, subjects received an intravenous injection of either 0.15 M NaCl (control) or CCK (GIH Research Unit, Karolinska Institutet, Stockholm, Sweden; 20% pure.). The CCK dose was 0.5 Ivy dog units kg^{-1}, given over 30 s. Two to six days, during which only NaCl injections were used, were allowed for familiarisation with the procedure. After this period, the next two days were used for collection of the data reported. CCK and saline were injected in random order on these two days. The study design was double-blind. Subjects were not aware that CCK was injected only on one of the last two days.

The infusion experiment differed only in that infusion of either CCK, 3 units kg^{-1} h^{-1} in 0.15 M NaCl, or NaCl alone, was begun 20 min before the subject started to eat, and continued for the 20-min eating period. Thus, each subject received a total of 1 unit kg^{-1} before, and 1 unit kg^{-1} during, eating.

After rapid injection, CCK decreased food intake ($P<0.025$, Table 1). The mean decrease was 54 ml (57 kcalorie; 12%). Almost all (96%) of the decrease occurred in the first 8 min (Fig. 1). During infusion, CCK increased food intake ($P<0.05$, Table 2). The mean increase was 113 ml (120 kcalorie; 22%). An increase occurred in four of the five 4-min periods (Fig. 1).

Subjects could not distinguish CCK injections or infusions from those of NaCl. No side-effects occurred during the eating period. Within 30–60 min after the CCK infusion was stopped, three subjects had 1–3 small loose stools (Table 2).

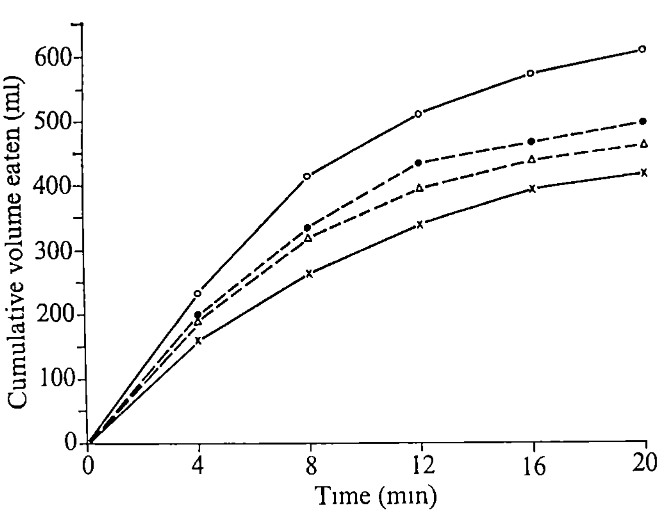

Fig. 1 Cumulative mean volume of Ensure (1.06 kcalorie m^{-1}) eaten during CCK (○, 3 units kg^{-1} h^{-1}) infusion (6 subjects) or after rapid CCK (×, 0.5 units kg^{-1}) injection (10 subjects). Control (0.15 M NaCl) data from the same subjects: ●, infusion control; △, injection control Cumulative volume eaten at 20 min was significantly greater during CCK infusion ($P < 0.05$), and significantly less after CCK injection ($P < 0.025$), than with the corresponding control treatments.

Table 1 Volume (ml) eaten after injection of NaCl control or 0.5 Ivy dog unit kg^{-1} CCK

Subject	NaCl (ml)	CCK (ml)	Difference (ml)
1	770	660	−110
2	660	560	−100
3	580	540	− 40
4	520	570	+ 50
5	460	360	−100
6	440	440	0
7	410	410	0
8	360	230	−130
9	290	240	− 50
10	180	120	− 60
Mean	467	413	− 54/ml

$P < 0.025$ (paired t test)

Diarrhoea did not occur on the control days. Side-effects did not occur in the 24 h after CCK injection. All subjects were satiated within 20 min of beginning each meal. One (injection) subject stated that the meal was not enjoyable, but she had the second largest mean intake.

The amount of inhibition in the injection study was considerably less than in the animal studies[1–4]. The most obvious explanation is that our CCK dose was substantially smaller than previous doses[1–4]. For example, in rhesus monkeys 5 units kg^{-1} produced a 26% decrease in food intake, whereas 20 units kg^{-1} produced a 70% decrease. We attempted to test 1 unit kg^{-1}, but side-effects (nausea, abdominal cramping) occurred. Although mild and transient, these effects were sufficient to destroy the double-blind design, and thus hopelessly confounded interpretation of the effects of that dose.

The infusion CCK dose was chosen to quantitatively reproduce effects on pancreatic secretion and gall bladder contraction ascribed to CCK during normal digestion[6]. Thus, it might be considered a 'physiological' dose[7].

The occurrence of loose stools shortly after stopping the infusion suggests, however, that physiological blood concentrations were exceeded (perhaps in conjunction with endogenously released CCK), resulting in an increased rate of transit of lumenal contents through the small bowel[8]. CCK blood concentrations were not determined, since no reliable method for their quantitative assay was available.

The CCK preparation used was only 20% pure, so the

Table 2 Volume (ml) eaten during infusion of NaCl control or 3 Ivy dog units kg^{-1} h^{-1} CCK

Subject	NaCl (ml)	CCK (ml)	Difference (ml)
1*	680	920	240
2*	660	900	240
3	530	540	10
4*	460	480	20
5	410	530	120
6	230	280	50
Mean	495	608	113

$P < 0.05$ (paired t test)

*1–3 loose stools after CCK.

possibility that either or both of the effects on food intake were due to impurities cannot be ruled out. In rats and monkeys, however, inhibition of food intake by CCK (from the same source as our CCK) was reproduced by the synthetic octapeptide of CCK, indicating that CCK, rather than impurities, produced the inhibition in the animal studies[1,4].

In summary, either stimulation or inhibition of food intake has been produced by CCK in man, depending on the dose and duration of administration. Further experiments are necessary to clarify the mechanism(s) of these effects, and their relationship to effects produced by endogenously released CCK during normal digestion.

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Methanol poisoning in the folate-deficient rat

THE recent attention given to methanol as a fuel has reawakened interest in its biological liability. Its toxicity is well known[1]. Methanol poisoning is characterised in man by a latent period of 12–24 h followed by metabolic acidosis, ocular toxicity and death. This syndrome has been reported by Gilger and Potts[2] to occur in the rhesus monkey, but Cooper and Felig[3] were unable to repeat this work. Recent studies have tended to confirm the results of Gilger and Potts. McMartin *et al.*[4] and Clay *et al.*[5] have shown the importance of formate generation and accumulation in the development of metabolic acidosis in the monkey, and our laboratory has been able to show ocular toxicity in the rhesus monkey[6]. On the other hand, formate does not accumulate in the rat[4] and methanol poisoning as described for man does not occur in the rat or other small laboratory animals[1,2].

Palese and Tephly[7] have shown that formate oxidation to CO_2 *in vivo* is dependent on a folate one-carbon pool pathway. Although Herken *et al.*[8] have reported formate accumulation in blood and acidosis in the dog after methanol and amethopterin treatment, the blood *p*H changes were minimal ($-\Delta p$H of 0.09 at most).

We report here the development of a profound acidosis in the rat coincident with the accumulation of high levels of blood formate after methanol treatment of folate-deficient rats. Male Sprague–Dawley rats (180–210 g) were divided into two groups fed either a folate-deficient diet or an appropriate control diet (Bio-Serve, Frenchtown, New Jersey). Liver folate levels were determined by a radioassay adapted from the method of Chanarin *et al.*[9] with the use of kits obtained from Diagnostic Biochemistry, San Diego, California. Formate measurements were made on tail vein samples by the method of Makar *et al*[10] and $^{14}CO_2$ measurements were made as described by Palese and Tephly[7]. Blood *p*H was determined on samples obtained by cardiac puncture using a blood gas analyser (Instrumentation Laboratories, Model No. 713).

Table 1 Effect of folate deficiency on ^{14}C-methanol oxidation in the rat *in vivo*

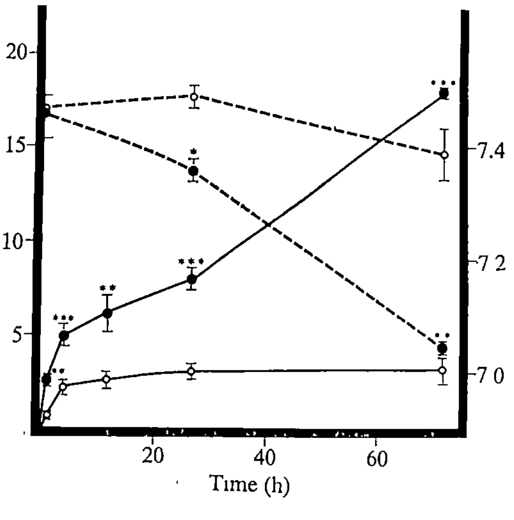
Time after methanol administration (h)	^{14}C-methanol oxidation (% dose) Control	Folate eficiency
4	2.62±0.18	1.51±0.15
8	4.28±0.24	2.43±0.21
12	5.58±0 33	3.15±0.22
20	8.37±0 40	4 33±0.28
27	9.83±0.51	5.07±0.33

Rats received ^{14}C-methanol (4 g kg^{-1}) intraperitoneally. Each value represents the mean of four observations±s.e.

Table 1 shows the rate of ^{14}C-methanol oxidation to $^{14}CO_2$ in control and folate-deficient rats. The amount of methanol recovered as $^{14}CO_2$ was decreased to about 50% in rats fed the folate-deficient diet. Levels of liver folate were 10.26±0.63 (s.e.) and 2.33±0.28 (s.e.) μg per g tissue in control and folate-deficient rats, respectively. These data correlate well with the results of Palese and Tephly[7], where liver folate levels and formate oxidation to CO_2 were reduced to a similar extent in folate-deficient rats. Figure 1 shows results for accumulation of formic acid in the blood and blood *p*H in animals fed on control and folate-deficient diets and which had received methanol (4 g kg^{-1}). A marked formic acidaemia and acidosis ($-\Delta p$H of 0 42, maximally) was observed in the group fed a folate-deficient diet. These results represent the first definitive demonstration of acidosis after methanol treatment in the rat, and the magnitude of changes resemble those already reported[4] for the rhesus and pigtail monkey.

These results indicate the importance of the folate-dependent one-carbon pool in formate metabolism and its probable role in methanol poisoning. The variability in sensitivity to methanol which has been suggested for man[11] may be explained by the relative state of folate deficiency and functional activity of the one-carbon pool pathway. Furthermore, the species differences observed between primates and the rat may be related to this nutritional component and its biological disposition. Finally, in certain

Fig. 1 Blood formate levels (—) and *p*H values (- - -) of control (○) and folate-deficient rats (●). At zero time, methanol was administered at a dose of 4 g kg^{-1} intraperitoneally. Each point represents the mean value obtained from 2–8 rats Vertical bars represent±s.e.; *, ** and *** indicate statistically significant differences from corresponding control values at *P* levels of <0.05, <0.01, and <0.001, respectively.



circumstances the folate-deficient rat may be a useful model for certain aspects of study on methanol poisoning.

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Frequency dependence of cyclic AMP in mammalian myocardium

Although there is now good evidence that cyclic AMP is a mediator of positive inotropic effects of catecholamines in the heart, the details of the metabolism of this nucleotide in the intact myocardial cells are still unknown. It has been shown that the intracellular concentration of cyclic AMP oscillates during each contraction cycle of the frog heart[1,2]. But little information is available for the influence of frequency of stimulation, which regulates myocardial contractility in physiological conditions[3], on the intracellular level of cyclic AMP. We therefore investigated the relationship between cyclic AMP level and the strength of contraction of the ventricular myocardium during the induction of different contractile states by changing frequency of stimulation. In order to analyse the influence of frequency of stimulation on cyclic AMP level, the effects of changes in concentrations of extracellular calcium ions or of D600, which is thought to inhibit the influx of calcium ions through the myocardial cell membrane[4], were also investigated. It was found that the intracellular level of cyclic AMP and the contractile force of isolated rabbit papillary muscles had a reciprocal relationship during induction of different contractile states by changing frequencies of stimulation. The influence of frequency was simulated by reduction of the extracellular calcium ions or by D600.

Experiments were carried out with papillary muscles isolated from the right ventricle of rabbits of both sexes (1.3–1.9 kg). Experimental methods were the same as previously reported[5]. The papillary muscles were mounted in a 20-ml organ bath containing Krebs–Henseleit solution (NaCl, 118 mM; KCl, 4.7 mM; $CaCl_2$, 2.55 mM; $MgSO_4$, 1 18 mM; KH_2PO_4, 1.18 mM; $NaHCO_3$, 24.9 mM; glucose, 11.1 mM) equilibrated with 95% O_2 and 5% CO_2 at a temperature of 37 °C. The final tension of the muscles was recorded isometrically through a force displacement transducer on an ink-writing oscillograph. The papillary muscle equilibrated in the Krebs–Henseleit solution was frozen immediately in liquid nitrogen and weighed, and the content of cyclic AMP was determined by the protein binding method of Gilman[6] as modified by Schwabe and Ebert[7]. The time required for freezing was 5–8 s.

Figure 1 shows the cyclic AMP level and the final tension of the papillary muscles stimulated at different frequencies. The tension of the papillary muscle stimulated at a rate of 1 Hz for 1 h was 416 ± 23 mg (mean ± s.e.; $n=4$). It was stable during the 60-min experimental period. When the frequency of stimulation was reduced to 0.5, 0.22, 0.1 and 0.05 Hz or elevated to 2 and 3 Hz, the final tension decreased to an extent dependent on the frequency, according to the well known frequency–force relationship for ventricular muscle. The cyclic AMP level after an equilibration time of 1 h at 1 Hz amounted to 0.51 pmol mg^{-1} wet weight of the tissue, which was stable for a further 60 min if the frequency was not changed. In contrast to the final tension the cyclic AMP level in papillary muscles was significantly high at 0.1 Hz ($P<0.001$ compared with the value at 1 Hz) and became lower as the frequency was raised to 1 Hz. At frequencies higher than 2 Hz, the cyclic AMP level increased again and amounted to 0.72 pmol mg^{-1} wet weight at a rate of 3 Hz ($P<0.05$ compared with the value at 1 Hz). Thus, the cyclic AMP level of papillary muscle changed in a reciprocal relationship to the strength of contraction during stimulation at different frequencies.

Table 1 shows the influence of changes of the extracellular calcium ion concentration on cyclic AMP level and final tension of papillary muscles stimulated at 1 Hz. When the extracellular calcium ion concentration was reduced from the level of the Krebs–Henseleit solution (2.55 mM) to 0.25 or 0, respectively, the tension decreased and the cyclic AMP level rose. Treatment of the muscle with 10^{-6} M D600 for 30 min caused an effect similar to that of reducing the extracellular calcium ion concentration· a significant increase in cyclic AMP and a decrease in tension.

It has been shown that the uptake of radioactive calcium ions per beat increases proportionally with the strength of contraction when the strength of contraction is varied by changing the frequency of stimulation[8]. Our observations are therefore compatible with the view that the intracellular concentration of cyclic AMP, as well as the strength of contraction, may be regulated by intracellular free calcium ions. This view is reinforced further by the finding that the reduction of intracellular calcium ions probably

Fig. 1 Influence of frequency of stimulation on cyclic AMP level and tension of the isolated papillary muscle of rabbits. Papillary muscles were stretched by a tension of 0 5 g and driven electrically by a square wave pulse of 5 ms duration and a voltage about 20% above threshold at different frequencies through platinum electrodes by the use of a Hugo Sachs Elektroniks Stimulator I. Muscles were equilibrated for 1 h at a frequency of 1 Hz before the frequency was adjusted to an experimental rate. The cyclic AMP level and the tension of the papillary muscle were determined when muscle tension reached the new steady level at each frequency. Numbers refer to number of papillary muscles at each point of the cyclic AMP level Number at the right of the curve for the muscle tension represents the number of papillary muscles used for the determination of the entire course of the frequency–force relationship. ○, Muscle tension; ●, Cyclic AMP.

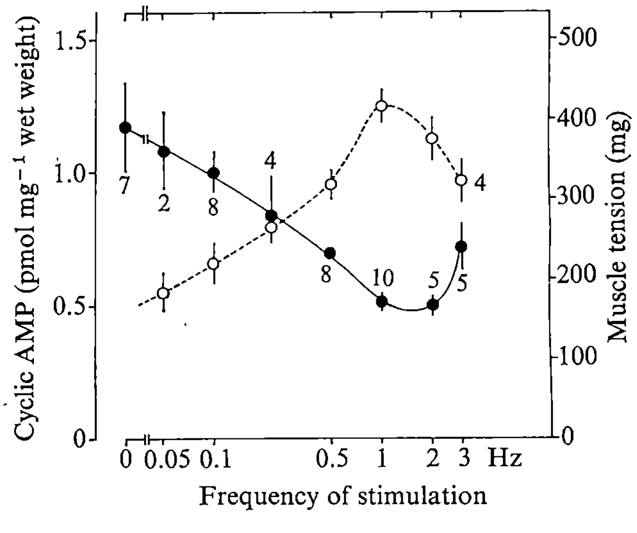

Table 1 The content of cyclic AMP and the developed tension of the rabbit papillary muscle in different conditions

		Cyclic AMP (pmol mg^{-1} wet wt)	Muscle tension (% of control)
Ca^{2+}	0* mM	0.95±0 09 (7)†	6 2±0.8 (7)†
	0.64 mM (×1/4)	0 85±0.19 (12)	17 9±4 1 (7)†
	2 55 mM (normal)	0 51±0.04 (10)	100
D600	10^{-6} M (Ca^{2+} 2.55 mM)	0.86±0.07 (10)†	17 9±3.5 (10)†

Influence of changes in extracellular calcium ion concentration and of D600 on cyclic AMP level and tension of isolated rabbit papillary muscle stimulated electrically at a rate of 1 Hz. The cyclic AMP level of the muscle was determined when muscle tension reached the steady level in the new experimental conditions, that is, approximately 30 min.

*No calcium was added to the medium.

†$P < 0.001$ compared with the control value in the normal calcium concentration

induced by reducing the extracellular calcium ion concentration or by D600, caused a similar reciprocal change, that is, the elevation of the intracellular level of cyclic AMP and the reduction of the strength of contraction.

An increase in cyclic AMP in myocardial cells induced by catecholamines is fast and short-lived: the intracellular concentration of cyclic AMP returns rapidly to the control value before the peak response of contraction is reached[9]. This rapid reduction of the intracellular cyclic AMP by contrast with the gradual inotropic action of catecholamines suggests an intracellular mechanism for regulating the intracellular level of the nucleotide in the myocardium. It has recently been demonstrated that low concentrations of calcium ions stimulate phosphodiesterase from bovine hearts[10] and inhibit adenylate cyclase from guinea pig hearts[11] in cell-free enzyme preparations. A similar regulatory role of calcium ions on the intracellular concentration of cyclic AMP is proposed also in the central nervous system[12,13]. In the present study, however, the possibility that the increased energy requirements for tension development at higher frequencies of stimulation led to the reduction of cyclic AMP must be also considered.

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Antipsychotic drug doses and neuroleptic/dopamine receptors

ANTIPSYCHOTIC drugs, or neuroleptics, are thought to act by blocking dopamine receptors in the nervous system[1–4]. Recent direct evidence, based on stereospecific binding assays, supports this hypothesis of antipsychotic drug action[5–9]. As only a few antipsychotic drugs had been tested for their effects on the binding of haloperidol[5–8], the question remained whether all antipsychotic drugs, regardless of chemical structure, would block the stereospecific binding of haloperidol. We report here that all clinically effective antipsychotic drugs (tested so far) block the stereospecific binding of ^{3}H-haloperidol at concentrations which correlate directly with the clinical potencies.

Binding of ^{3}H-haloperidol was done on calf caudate homogenates, while binding of ^{3}H-dopamine was done using rat caudate, by modifications of methods described before[5–7]. The fresh caudate tissues were homogenised in 10 volumes of ice-cold TEAN buffer (15 mM Tris-HCl, *p*H 7.4, 5 mM Na_2EDTA, 1.1 mM ascorbate and 12.5 μM nialamide), using a Teflon–glass homogeniser (0.16 mm clearance, 500 r.p.m.; 15 up–down strokes). This homogenate was incubated at 37 °C for 1 h, subdivided into 200 mg wet tissue per vial, and stored frozen at −20 °C for periods of up to 3 months. Immediately before the assay, the 200-mg sample was thawed and centrifuged (15 min at 44,000*g*) at 4 °C. The supernatant was discarded and the pellet was resuspended in 5 ml of TEAN buffer, using a ground-glass homogeniser (five strokes by hand). The homogenate was then homogenised further using a Brinkmann Polytron (PT-10) at a setting of 7.0 (full scale = 10) for 20 s. The final protein concentration of the suspension was 1 1 mg ml^{-1}. For the binding assay, samples were added to a glass test tube (12×75 mm) as follows: 100 μl of 600 nM (+)-butaclamol or (−)-butaclamol (the final butaclamol concentration was 100 nM in the ^{3}H-haloperidol assay, but 1 μM in the ^{3}H-dopamine assay[7]); 100 μl of buffer containing different concentrations of antipsychotic drugs; 200 μl of ^{3}H-haloperidol (6.4 nM, 9.66 Ci mmol^{-1}) or ^{3}H-dopamine (3.5 nM, 8.4 Ci mmol^{-1}); and 200 μl of membrane suspension. Although the final total concentration of ^{3}H-haloperidol was 2.14 nM, more than 50% of the drug becomes adsorbed (the drug is very membrane-soluble[10,11]) and so the free concentration (that is the concentration of drug not bound) was actually 0.97 nM in all experiments. After incubation at room temperature (20–21 °C) for 30 min, 0.5 ml of the mixture was filtered (Whatman GF/B glass fibre filter, 2 4 cm), using a 600-mmHg vacuum, followed by washing with 5 ml of ice-cold TEAN buffer The filters were monitored for ^{3}H as described before[5–7]. The stereospecific component of binding was defined as that amount of ^{3}H-haloperidol or ^{3}H-dopamine bound in the presence of (−)-butaclamol (inactive neuroleptic) minus that bound in the presence of (+)-butaclamol (active neuroleptic). Each experiment was done in sextuplicate, and each antipsychotic drug was tested at least three times, with most tested nine times.

At the usual C_{free} of 0 97 nM ^{3}H-haloperidol, the stereospecific component of haloperidol binding was 90 fmol per mg protein of calf caudate homogenate A Scatchard analysis (J Tedesco and P. S., unpublished) indicated the total number of sites to be 14 pmol per g of wet tissue.

At the usual C_{free} of 0.97 nM ^{3}H-haloperidol, the stereo-^{3}H-haloperidol The IC_{50} values (concentrations producing 50% blockade of haloperidol binding) are shown in Fig. 1 plotted against the average clinical doses used for controlling schizophrenia (refs for doses are in refs 12 and 13).

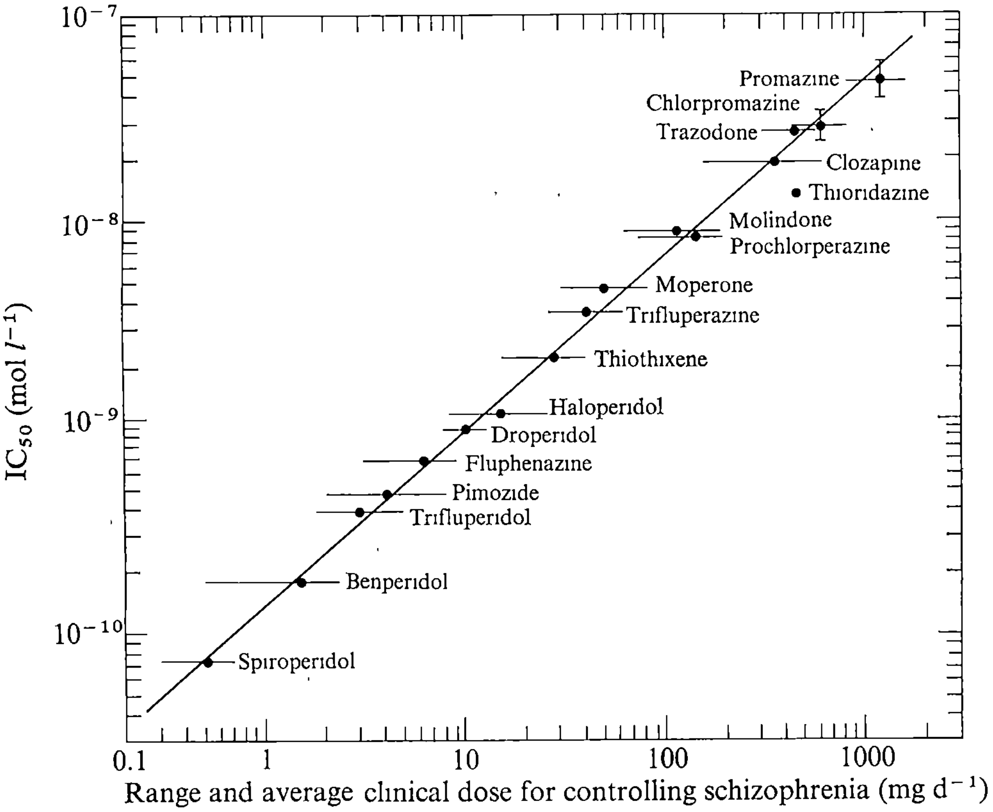

Fig. 1 The IC_{50} values (ordinate) are the concentrations of the antipsychotic drugs which reduce the stereospecific component of ^3H-haloperidol binding by 50%. The abscissa indicates the average values (and ranges) of doses used for schizophrenia.

The s.e.m. for each IC_{50} is less than 10% of the IC_{50}. Further IC_{50} values for ^3H-haloperidol and ^3H-dopamine are

The results in Fig. 1 indicate that there is a good correlation between the antipsychotic potencies and the IC_{50} values for blocking the neuroleptic receptor The neuroleptic receptor assay can thus be used to screen potentially useful antipsychotic drugs. The antipsychotic drugs also block the binding of ^3H-dopamine, and the IC_{50} values for this effect correlate qualitatively, with the clinical antipsychotic potencies, but more data are needed on this point.

The IC_{50} values may be of therapeutic value since they are almost identical to the concentrations of the antipsychotic drugs in the plasma water of the patient. For example, in the case of haloperidol being administered to a patient at doses of about 15 mg d^{-1} the plasma concentration of haloperidol is approximately 5 ng ml^{-1} (ref. 14). As about 10% of this is free haloperidol (unpublished data), the concentration of free haloperidol in the patient's plasma water is about 1.3 nM; this compares with the IC_{50} value of 1 2 nM. In the case of chlorpromazine, the plasma concentration is of the order of 200 ng ml^{-1} (after dose corrections[15,16]); because 95% of the chlorpromazine is bound[10], the free concentration of chlorpromazine in the plasma water of the patient would be of the order of 30 nM. This compares with an IC_{50} of 29 nM for chlorpromazine acting on the neuroleptic receptor (Fig. 1).

Table 1 IC_{50} values for ^3H-haloperidol and ^3H-dopamine

	^3H-haloperidol IC_{50}	^3H-dopamine IC_{50}	
	calf (nM)	calf (nM)	rat (nM)
Dopamine	600	9	
Apomorphine	40	2	45
Promethazine	2,000		
Melperone	50		
Chlorpromazine	29	2,000	420
Clomacran[17]	28		
Clozapine	20		200
Metiapine	15		
Molindone	9		150
Lenperone[18]	20		
Haloperidol	1.2	300	35
Fluphenazine	0.7		30
cis-Piflutixol*	0.4		
trans-Piflutixol*	300		
p-Fluoro-spiroperidol†	0 05		

*Personal communication from I Moller Nielsen.
†Personal communication from P. J Janssen.

Finally, although the antipsychotic drugs are active in both the neuroleptic and dopamine radioreceptor assays, and although dopamine and apomorphine are active in both assays, this does not prove that the two receptors are identical, particularly since the IC_{50} values are not identical in the two assays. The agonist and antagonist ligands may also hold the receptor in a different conformation state.

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Proposed structure for brain adenylate cyclase purified using blue dextran–Sepharose chromatography

We have recently shown[1] that blue dextran–Sepharose affinity chromatography can be used to advantage to abbreviate markedly the purification procedures for soluble enzymes whose nucleoside phosphate binding sites are constructed by a supersecondary structure called the NAD-domain. Preliminary measurements using lactate dehydrogenase indicated that affinity chromatography with blue dextran–Sepharose can also be done in the presence of various non-ionic detergents used to solubilise membrane proteins. Here we describe the use of blue dextran–Sepharose affinity chromatography for the purification of the membrane-bound hormone-responsive enzyme, adenylate cyclase, and propose a new structure for the membrane complex which does not contain a separate hormone receptor protein.

Frozen bovine brain cortex was homogenised and the insoluble material extracted with 2% Lubrol PX as described by Johnson and Sutherland[2]. All solutions contained 5 mM NaF and 5 mM $MgCl_2$ and all operations were done at 4 °C to stabilise the enzymatic activity. The centrifuged Lubrol extract obtained from 1 kg of cortex was stirred overnight with 100 ml of blue dextran–Sepharose prepared by the procedure of Ryan and Vestling[3]. The blue dextran–Sepharose was filtered into a chromatographic column and washed with a litre of 10 mM Tris-HCl buffer, *p*H 7.5 containing 0.1 mM ATP. The adenylate cyclase activity was then eluted with 250 ml of the Tris buffer containing 1 mM ATP. This eluate was applied to a DEAE–cellulose chromatographic column having a bed volume of 100 ml and equilibrated with 10 mM Tris-HCl buffer, *p*H 8.0. The column was washed with the equilibration buffer and then eluted with a linear 0–1 M NaCl gradient contained in 500 ml of the equilibration buffer. The adenylate cyclase activity emerged as a symmetrical peak centred at about 0.9 M NaCl. Fractions containing enzymatic activity were pooled, dialysed and concentrated using an Amicon XM-50 membrane.

Results of a typical purification are given in Table 1. The specific activity of the Lubrol-extracted enzyme solution is within the range reported for detergent-extracted adenylate cyclase from cat heart[4] and rat brain[2] preparations. As shown in Table 1, about 50% of the adenylate cyclase activity in the Lubrol extract of bovine brain is retained by and eluted from the blue dextran–Sepharose affinity columns. This value can be increased to 65% by performing the chromatography in solvents containing 0.1 mM noradrenaline. The remaining 35% of the extracted enzymic activity does not bind upon reapplication to the affinity column. We assume this represents particulate bound enzyme since increasing the concentration of Lubrol increases the amount of enzymic activity retained by the affinity column. Higher concentrations of Lubrol solutions were not used, however, because of their high viscosity and resultant slow flow rates. Lubrol can be omitted from all solutions after initial solubilisation of the enzyme without adversely affecting the retention of catalytic activity.

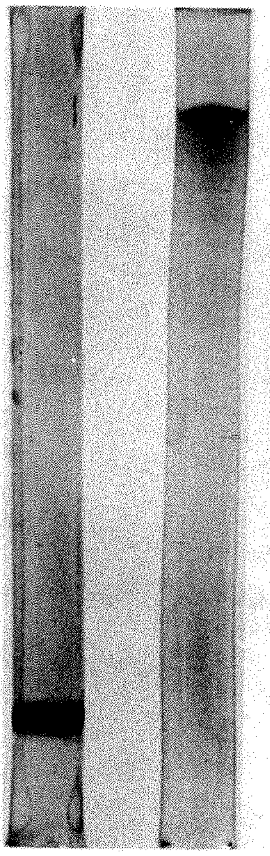

Fig. 1 Zone electrophoresis of bovine brain adenylate cyclase in polyacrylamide gels. Samples of the pooled enzymically active proteins obtained from Sephadex G-200 exclusion chromatography were applied to the top of each gel as shown. The right gel was maintained at *p*H 8.9 while the left gel contained 1% sodium lauryl sulphate. Electrophoresis was done for 2 h using 3 mA per gel. Both gels were stained with Coomassie brilliant blue.

About 85% of the total protein and 100% of the total catalytic activity of the material obtained by DEAE–cellulose chromatography was eluted from a calibrated Sephadex G-200 exclusion chromatographic column as a single symmetrical peak at a position corresponding to a molecular weight of $1.14 \pm 0.09 \times 10^5$ for a cytoplasmic globular protein. As shown in Fig. 1, the pooled fractions from exclusion chromatography containing enzymic activity migrate as a single component after polyacrylamide gel electrophoresis both at *p*H 8.9 using the procedure of Davis[5] and in 1% sodium dodecylsulphate using the procedure of Weber and Osborn[6]. The mobility of the single

Table 1 Purification of adenylate cyclase from bovine brain cortex

Step	Volume (ml)	Total protein (mg)	Specific activity (nmol min^{-1} mg^{1-})	Yield (%)
Lubrol extract	4,500	4,210	0.097	100
Blue dextran–Sepharose chromatography	30	14	14.5	50
DEAE cellulose chromatography	35	3.5	23.6	20

Aliquots of enzyme solutions were incubated for 15 min at 37 °C in 1 mM ATP (α-^{32}P, about 5m Ci mmol^{-1}), 10 mM theophylline, 5 mM NaF, 5 mM $MgSO_4$ and 40 mM Tris-HCl buffer, *p*H 7.5. The catalysed reaction was terminated by rapidly heating to 100 °C. The product of the catalysed reaction, cyclic AMP, was purified by neutral alumina chromatography as described by Ramachandran[9] and the concentration of cyclic AMP measured by liquid scintillation spectrometry. No corrections have been made in the adenylate cyclase activity measurements for the presence of theophylline-insensitive phosphodiesterases. Protein concentration was measured by the procedure of Lowry using 0.1% sodium lauryl sulphate to solubilise any Lubrol PX present[10]. Bovine serum albumin was used as the protein standard.

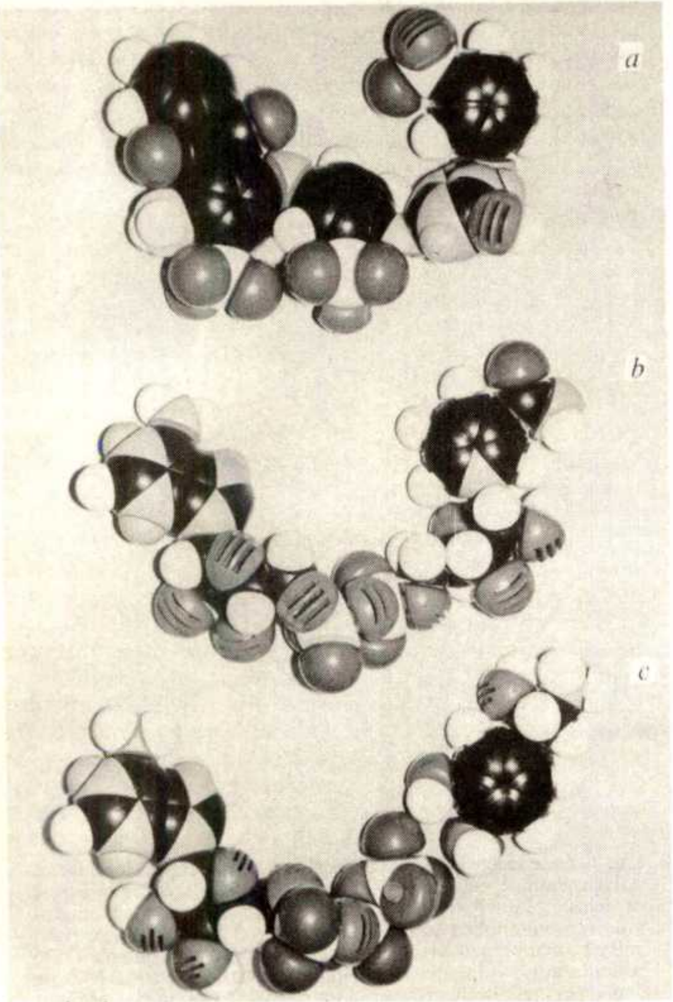

Fig. 2 Comparison of Corey–Pauling–Koltun (CPK) models of Cibacron blue F3GA (*a*), NAD (*b*) and ATP and noradrenaline (*c*).

component in detergent corresponds to a polypeptide molecular weight of $1.59 \pm 0.18 \times 10^4$. The catalytic activity of both the purified enzyme and the enzyme in the initial Lubrol extract is maximally stimulated twofold in the presence of 5 mM NaF and fourfold in the presence of 100 μM of the catecholamines noradrenaline or dopamine. By contrast, the catalytic activity is not stimulated by the presence of up to 1 mM of adrenalin, seretonin, γ-amino butyrate or acetylcholine at any stage of the enzyme purification. This selective response of solubilised adenylate cyclase to hormones and neurotransmitters parallels the response of the rat brain enzyme in synaptosomal fractions[7]. The presence of both 5 mM NaF and 5 mM $MgSO_4$ is required to stabilise the catalytic activity of the bovine brain adenylate cyclase throughout the purification procedure. The purified enzyme is stable for at least 8 weeks when stored in 5 mM NaF at *p*H 7.5 and 4 °C. Since the presence of theophylline does not increase the catalytic rate of the purified enzyme, it must be free of phosphodiesterase activity.

The properties of purified adenylate cyclase suggest that the enzyme in aqueous solution contains eight identical polypeptide chains each having a molecular weight of 16,000. Since no larger catalytically active forms of the enzyme are detected, it is likely that the polypeptide chains form globular subunits arranged in a cubic quaternary structure, two subunits wide in each dimension. Assuming the protein has a $\bar{v}$ of 0.75 ml g^{-1}, the cubic quaternary structure would measure 68 Å in each dimension, a distance sufficient to span the plasma membrane.

Since adenylate cyclase binds specifically to the blue dextran–Sepharose affinity columns, we propose that each subunit contains an NAD-domain which forms the binding site for the substrate ATP. Indeed, since formation of a typical NAD-domain requires about 150 amino acid residues[8], the tertiary structure of each subunit must entirely consist of an NAD-domain. Comparison of CPK models for the blue chromophore of blue dextran, Cibacron F3GA, of NAD as it binds to the NAD-domain of dehydrogenases and of ATP and noradrenaline, shown in Fig. 2, indicates that the dinucleotide fold of adenylate cyclase could easily bind both the substrate ATP and the hormone in adjacent positions. As shown in Fig. 2, the ATP is oriented as the ADP portion of NAD whereas noradrenaline is oriented as the nicotinamide portion of NAD maintaining similar positions for the side chain amide of nicotinamide and the ethanolamine side chain of noradrenaline. This orientation of noradrenaline and ATP would facilitate hydrogen bonding between the two phenolic hydroxyl groups of noradrenaline and the terminal phosphate of ATP which could enhance the rate of formation of cyclic AMP in solution. Alternatively, occupancy of nicotinamine portion of the NAD-domain could promote a conformational change which would enhance the rate of catalysis.

The low concentration of ATP in the brain extracellular fluid and the absence of catecholamines in the intracellular fluid, however, precludes ligation of both ATP and catecholamine on the same NAD-domain when the enzyme is located within the plasma membrane. Accordingly, we propose that ligation of noradrenaline or dopamine to the NAD-domain of one or more of the subunits of the plasma membrane enzyme exposed to the extracellular fluid produces a conformational change which accelerates the rate of catalytic conversion of ATP to cyclic AMP at the NAD-domains of the subunits facing the intracellular fluid. Thus, a membrane bound oligomeric protein containing identical subunits could function as an allosteric enzyme without requiring a separate hormone receptor protein. Experimentation is currently being conducted to test these proposals.

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Synaptic transmission and effects of temperature at the squid giant synapse

THERE have been several reports on the effects of temperature on transmitter release at synaptic junctions[1–6]. Although all reports agree that synaptic transmission is affected by temperature, the relationships are often complex and may differ from one preparation to another. The preparations studied, however, have the disadvantage that the relationship of presynaptic events (such as spike amplitude) to postsynaptic response cannot be investigated. It is possible to study this relationship at the squid giant synapse where intracellular recording can be obtained

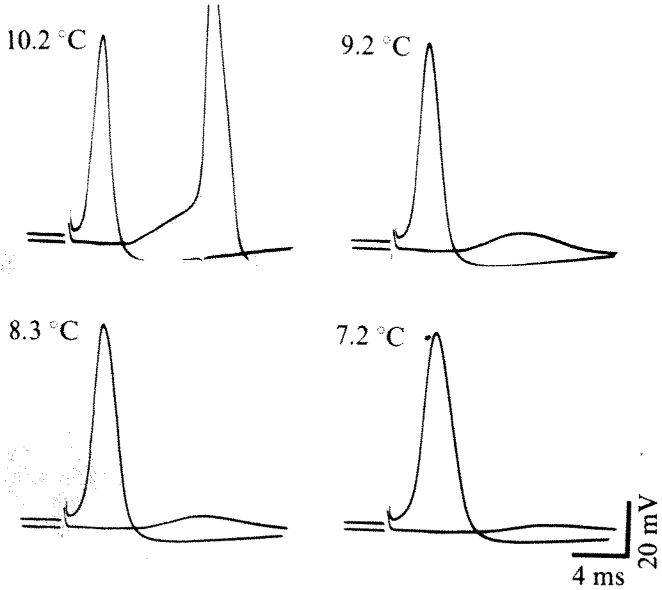


Fig. 1 Records of potentials in presynaptic terminal (upper trace) and postsynaptic axon (lower trace) of the squid giant synapse showing the effects of temperature (°C) on the presynaptic action potential and the e.p.s.p. The e.p.s.p. activates a postsynaptic action potential at 10.2 °C.

from both the presynaptic terminal and the postsynaptic axon. The excitatory postsynaptic potential (e.p.s.p.) at the squid synapse usually generates an action potential in the postsynaptic axon. To study the e.p.s.p., and hence transmitter release, without the postsynaptic action potential, tetrodotoxin has often been used [7–9]; this has not permitted study of the characteristics of the presynaptic spike in relation to the e.p.s.p. In a recent investigation on transmission at the squid giant synapse, we found that lowering the temperature of the preparation abolished activation of the postsynaptic action potential by the e.p.s.p., while the presynaptic spike was still generated. This observation enabled us to investigate the relationship between the presynaptic spike and the e.p.s.p. in various experimental conditions[10] and we report here the effects of changing temperature.

The stellate ganglion was dissected from the squid, *Loligo pealii,* placed in a small Lucite chamber and perfused continuously with oxygenated seawater[10]. The temperature of the bath was controlled by a Cambion thermoelectric device and monitored continuously by a thermometer (Tri-R Instruments). In this way, the bath could be maintained at a constant temperature (±0.1 °C) or the temperature could be changed rapidly. Micropipettes filled with 3M KCl were inserted in the mid-portion of the presynaptic terminal and in the postsynaptic axon near the synapse. Conventional electrophysiological recording techniques were used[10].

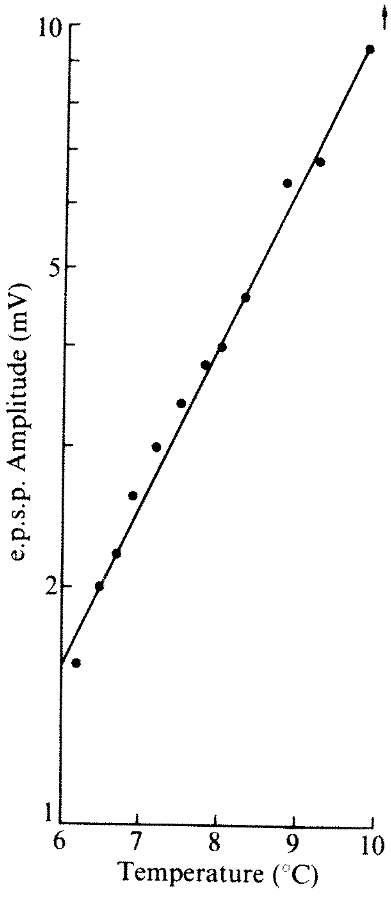
At room temperature (18–20 °C), the e.p.s.p. in the squid axon almost always generates a postsynaptic action potential. In all of the 43 synapses investigated, when the temperature was lowered sufficiently, initiation of postsynaptic spike generation by the e.p.s.p. did not occur. The mean temperature at which the e.p.s.p. failed to activate a spike was 8.9 °C±1.5 (s.d.m.) and the range was from 5.6 to 12 °C. One factor which may have a role in preventing the e.p.s.p. initiation of the postsynaptic action potential is an increase in the threshold of the action potential by the lowered temperature. Another factor which is probably more important, however, is a reduction in the e.p.s.p. amplitude produced by cooling.

Figure 1 shows the effect of lowering bath temperature on the e.p.s.p. and the presynaptic spike. At 10.2 °C (and higher temperatures) the e.p.s.p. initiated spike generation, but as the temperature was lowered, the postsynaptic spike was not activated and the e.p.s.p. amplitude decreased progressively. This was accompanied by an increase in the width of the presynaptic spike, due to a decrease in the rate of rise and fall of the action potential. This effect of temperature has been described previously for the action potential of the squid giant axon[11]. Finally, an increase in synaptic delay can also be seen, as reported previously[12].

Figure 2 shows a graphic representation of the change in e.p.s.p. amplitude as a function of temperature. There is a linear relationship between the log of the e.p.s.p. amplitude and temperature. The slope of the curve indicates a high degree of temperature sensitivity for transmitter release. From an Arrhenius plot of the log of the e.p.s.p. amplitude against the reciprocal of temperature (K), an apparent activation energy of 74 kcalorie per degree can be calculated. This compares with an activation energy of 25 kcalorie per degree reported[5] for transmitter release at the rat phrenic nerve–diaphragm synapse.

The relationships between e.p.s.p. amplitude, presynaptic spike height and temperature are plotted for comparison on a linear scale in Fig. 3. Figure 3*a* shows the relationship between e.p.s.p. amplitude and temperature; the slope of the curve is steepest in the temperature range between 9 and 10 °C. The relationship between presynaptic spike height and temperature is illustrated in Fig. 3*b*. In contrast to the curve relating e.p.s.p. amplitude to temperature, the presynaptic spike height curve has the greatest change in the range 6 to 8 °C. To assess whether the change in transmitter release at different temperatures is due to the effects of temperature on presynaptic spike height, the direct relationship between presynaptic spike height and e.p.s.p. amplitude at different temperatures is plotted in Fig. 3*c*.

Fig. 2 Graph of relationship between log of e.p.s.p. amplitude (mV) and temperature (°C) at the squid giant synapse. From the same experiment illustrated in Fig. 1.



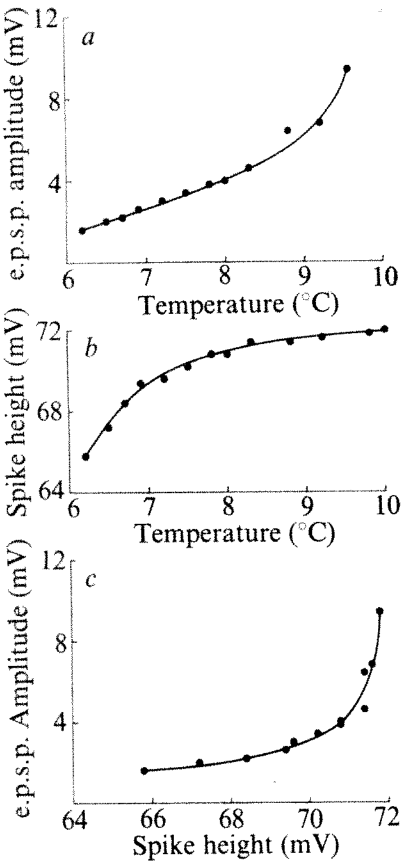


Fig. 3 Relationship between e.p.s.p. amplitude, presynaptic spike height and temperature (°C) plotted on linear coordinates. *a*, e.p.s.p. amplitude (mV) plotted as a function of temperature. *b*, Presynaptic spike height (mV) plotted as a function of temperature. *c*, Graphic relationship between e.p.s.p. amplitude (mV) and presynaptic spike height (mV) at different temperatures. From the same experiment illustrated in Figs 1 and 2.

This relationship does not agree with the relationship between presynaptic spike height and e.p.s.p. amplitude reported in previous studies using electrical polarisation of the presynaptic terminal[9,13–15]. This discrepancy suggests that changes in the presynaptic spike height may not be a major factor in the effect of temperature on transmitter release. The effect of temperature on transmitter release at the squid synapse is therefore likely to involve other factors such as temperature sensitivity of the release mechanism.

In summary, lowering the temperature of the squid giant synapse prevents e.p.s.p. initiation of the postsynaptic action potential and permits investigation of the relationship between the e.p.s.p. and the presynaptic action potential. An investigation of the effects of temperature on transmitter release reveals that transmitter release is temperature sensitive; from an Arrhenius plot an apparent activation energy of 74 kcalorie per degree, was calculated. Finally, a change in presynaptic spike height does not seem to be the major factor involved in the sensitivity of transmitter release to temperature.

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Stimulation of active Na transport by phospholipase C

WE have found that an enzyme, phospolipase C, which has a very specific action in cleaving phosphatidylcholine (lecithin) at its ester linkage (between the α carbon of glycerol and the phosphoryl group of phosphorylcholine) stimulates active Na transport across frog skin *in vitro*. This action may provide both insight into the sort of processes involved in transepithelial Na transport and a model for the action of proteins induced by aldosterone[1,2]. This hormone stimulates Na transport across epithelial membranes which, apart from the kidney tubules, include *in vitro* frog skin, and toad urinary bladder and colon[3–7].

When phospholipase C (Sigma, batch no. 84C-6871, Type I from *Cl. welchii*) was placed in the solution bathing the corium side of frog (*Rana pipiens*) skin, it produced a sustained increase in the short-circuit current (SCC), normally reflecting active Na transport, and transmural potential difference (Fig. 1). The peak effect was seen in about 30 min and the increase was maintained for several hours. When the enzyme was washed from the tissue, the SCC returned to the initial levels after about 1 h, suggesting that the membrane can reconstitute its original character. Phospholipase C placed on the epidermal side of the frog skin also produced an increase in the SCC, but its effect was much less. The activity of the phospholipase preparation was destroyed by immersing it in a boiling water bath for 10 min, which is consistent with its action being due to the enzyme. The SCC in the presence of phospholipase C was abolished by amiloride (10^{-4} M), which is a specific antagonist of active Na transport across such epithelia[8,9].

Cuthbert and Painter[10] have shown that phospholipase C produces an "initial stimulation" of SCC in frog skin, but this increase was followed by a prominent decline. To maintain the activity of the enzyme, they bathed the skin in non-aerated Ringer solution, a precaution which we found unnecessary, which probably accounts for the differences in the results. We have found that in the toad urinary bladder phospholipase C (on the serosal side) causes a small initial increase in SCC, which then declines. On the vitreous side of the toad crystalline lens it produced a sustained increase in the translenticular SCC.

Active Na transport across amphibian epithelia can be stimulated by several hormones and drugs, including antidiuretic hormone (ADH), aldosterone, and amphotericin B. The latter increases Na transport across the toad urinary bladder and it has been suggested to act analogously, and so serve as a model, to aldosterone[11]. Amphotericin B reacts with sterols in the walls of cells to create pores, or channels, through which Na$^+$ can pass. Its effect, however, cannot be inhibited by amiloride[9], and there is an increase in both the influx and efflux of Na$^+$ across the membrane[12]. In contrast aldosterone and ADH increase the influx but have little or no effect on the efflux.

We measured the effects of phospholipase C on the unidirectional fluxes of ^{22}Na, and found that while the influx increased there was no change in the efflux (Table

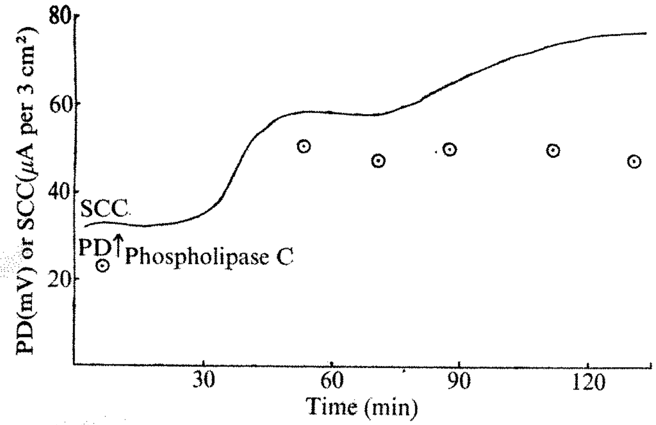

Fig. 1 Effects of phospholipase C (50 μg ml^{-1}, 8.5 U mg^{-1} on the corium side) on the SCC (μA cm^{-2}) and the potential difference (p.d.) (mV) across frog skin *in vitro*.

1). The SCC remained equivalent to the net Na transport. Thus phospholipase C acts specifically to increase Na influx, which is the direction of the active Na transport. Its effect is to create 'one-way' (amiloride-inhibitable) rather than 'two-way', nonspecific, channels in the membrane. The precise site of its action, however, is unknown, and it could be either increasing the access of Na to the pump mechanism or acting directly on the latter.

To explore further the relationships and analogies of the action of phospholipase C with that of natural hormones its effects were measured in the presence of ADH and aldosterone. The actions of vasotocin (the natural ADH in amphibians) and aldosterone appeared to be additive with those of phospholipase C (Table 2). This observation confirms the physiological integrity of the normal active Na transport mechanism in the presence of the enzyme. It also suggests that the precise sites involved in the responses to the hormones and the exogenous enzyme may differ.

These results are principally interesting with respect to the concept of possible mechanisms that may control active Na transport and account for its stimulation, such as by hormones like aldosterone. They show that an enzyme with a highly specific action can, reversibly, increase active Na transport by promoting influx without changing the efflux. Phosphatidylcholine could be the site of such an action and splitting of its ester linkage may be involved. This cleavage of the molecule would create free polar groups with which Na could interact, and this is in agreement with a proposed mechanism for transepithelial Na transfer in frog skin[13]. As it is unlikely that the phospholipase C enters the cells, it seems to be acting on their external surface. Such an effect could occur *in vivo* if an enzyme were secreted from specialised cells, such as the mitochondria-rich cells which have been proposed as a locus of hormonal action in the frog skin[14] and toad urinary bladder[2,15]. Such a secretory product could function as a lateral transmitter and stimulate Na transport in neighbouring cells. The results also suggest that proteins, such as those induced by aldosterone, that may stimulate active Na transport, could be similar to phospholipase C.

Table 1 Effects of phospholipase C on unidirectional fluxes of ^{22}Na and the potential difference and SCC across frog skin *in vitro*

	Initial	Phospholipase C present	Mean difference
Na influx (μEq cm^{-2} h^{-1})	1.02	1.45	0.43 ± 0.08*
PD (mV)	24	39	15 ±2.9*
SCC (μA cm^{-2})	22	36	14 ±2.1*
Na efflux	0.29	0.26	0.03 ± 0.03
PD	18	34	16 ±2.3*
SCC	20	37	17 ±2.4*

*$P < 0.01$ for mean difference.

The ventral abdominal skins (3 cm^2) were mounted in divided Ussing-type chambers and bathed on both sides, at 21–22 °C, with Ringer solution containing (mM): NaCl, 111; KCl, 3.35; CaCl$_2$, 2.7; NaHCO$_3$, 4.0 and glucose, 5.0. The solutions on both sides were aerated and the *p*H was 8.3. Apart from briefly reading the potential difference (p.d.), the skin was maintained in short-circuited conditions with an automatic voltage clamp. Each flux value represents the mean of four 15-min periods. The results are expressed as the mean difference ± s.e. for six experiments per unidirectional flux.

Table 2 Interactions in the responses of frog skin to phospholipase C, vasotocin and aldosterone

	SCC (μA cm^{-2})		
	Initial	Final	Increase
(1) Vasotocin			
Control, vasotocin alone	23	35	12 ± 1.5
Vasotocin in presence of phospholipase C	34	48	14 ± 3.1
(2) Aldosterone			
Control, phospholipase C alone	17	31	14 ± 2.5
Phospholipase C in presence of aldosterone	40	57	17 ± 0.8

The frog skins were prepared as described in Table 1. In experiment (1) phospholipase C (50 μg ml^{-1}) was added, and after a stable response was seen, vasotocin (10 mU pressor per ml in a maximal dose) was placed on the corium side and the maximum increase in SCC was recorded. The effect of vasotocin on a paired control preparation was measured at the same time. In experiment (2) paired skins were incubated for about 18 h, one in the presence of aldosterone (10^{-7} M, both sides) at which time a peak stable response (= initial) to the hormone was seen. The enzyme was then added to this preparation as well as to the control preparation and the succeeding maximal stable values recorded.

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Membrane retrieval at neurosecretory axon endings

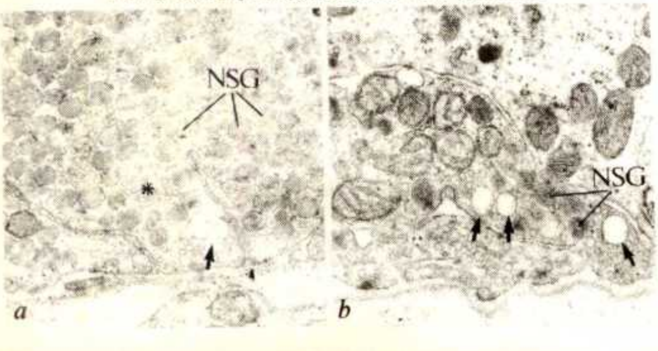
EARLY descriptions of neurohypophysial hormone release by exocytosis[1] suggested that, after exocytotic discharge of the content of the neurosecretory granules (NSG), the membrane of the granule was recaptured in the form of electron-lucent microvesicles of diameter ~ 50 nm. Such microvesicles were also shown to take up the extracellular marker horseradish peroxidase[2]. Larger vacuoles of a diameter similar to that of NSG have also been shown to take up this marker in stimulated neurohypophyses[3,4]. Furthermore, reuptake of membrane into organelles of approximately the size of NSG has also been predicted on the basis of studies of NSG-membrane labelling[5] and radioactive extracellular tracer uptake[4]. We have studied membrane retrieval after stimulation of the rat neurohypophysis by stereology[6,7] in an attempt to assess the relative contribution of vacuoles and microvesicles to the membrane reuptake process. Depletion of neurosecretory granules was associated with membrane retrieval by vacuoles of similar size. There was no detectable change in the microvesicle population.

Albino rats of 300–350 g body weight were decapitated, and the neural lobes were removed and incubated for 20 min in modified Locke's solution (150 mM NaCl, 2.2 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM $KHCO_3$, 10 mM glucose, *p*H 7.3) which was gassed continuously with 5% CO_2 in O_2. Glands were next incubated for 5 min in this solution where choline had been substituted for sodium. After this stabilisation period, the lobes were incubated for 15 min in either a high-potassium (56 mM K^+; 0 mM Na^+) Locke's solution to stimulate release of oxytocin and vasopressin, or in control (5.6 mM K^+, 0 mM Na^+) solution. The hormone released was assessed by measuring the oxytocin content of the medium, determined by a rat bioassay[8].

Point counting volumetry was performed on a sample of 201 profiles of nerve endings from six control glands and 207 profiles of endings from six stimulated glands (× 43,800). The endings were selected by reference to their position on the grid, and the micrographs assessed with the observer unaware of the source of the micrographs. For the purpose of this study a nerve ending was defined as a dilatation of a neurosecretory axon containing microvesicles in addition to NSG. Many profiles of such endings transected their contact zone with the basement membrane while others did not. Vacuoles were defined as electron-lucent vesicles more than 80 nm in diameter (Fig. 1*a* and *b*); their sectioned profiles were not always perfectly circular and, in stimulated glands (Fig. 1*b*), they occasionally contained a very small amount of electron-dense material. They differed from aldehyde-fixed NSG which had lost core electron density as a result of inappropriate fixation conditions[9] by virtue of their continuous trilaminar membrane and the electron lucency of their content, which was similar to that of the interior of microvesicles.

The results (Table 1) indicate that, in the endings, after stimulation of hormone release, the proportion of the total volume occupied by NSG is significantly ($P < 0.001$) decreased while that of vacuoles is significantly increased ($P < 0.001$). The vacuoles were significantly larger ($P < 0.001$) in stimulated than in control glands (Kolmogorov–Smirnov test) but this increase in size is insufficient to account for the increased volume that they occupy. The results therefore indicate that after release has been stimulated, the NSG decrease in number and the vacuoles increase both in number and in size (Fig. 2); by contrast neither the microvesicle (Fig. 2) nor the mitochondrial population was altered significantly. Furthermore, it should be pointed out that after hormone release there is no increase in the surface area of the endings. Depletion of NSG was greatest in those profiles of endings where contact with the basement membrane was included (Table 1), a finding consistent with exocytosis at this contact zone. Because, after stimulation, the vacuoles were found equally in profiles which did and did not transect the contact zone, it seems either that they are rapidly dispersed through the endings or that membrane reuptake does not occur exclusively at the release site. Such a rapid dispersal together with the low basal hormone turnover in the unstimulated neural lobe[10] would explain the paucity of vacuoles in endings from control glands.

Fig. 1 Electron micrographs of rat neurohypophyses (× 45,270). *a*, Control (5.6 mM KCl); *b*, stimulated neurohypophysis (56 mM KCl). The arrows indicate membrane-bounded vacuoles. *Microvesicles; NSG, neurosecretory granules.



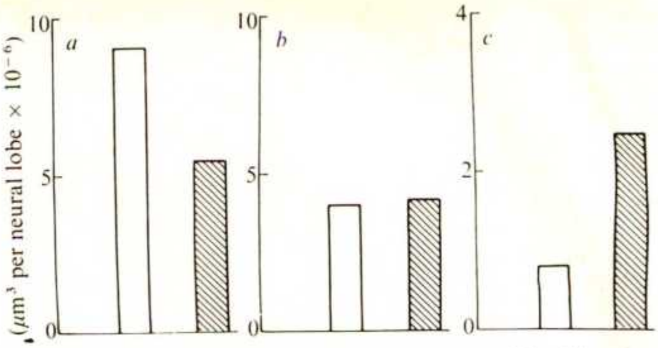


Fig. 2 Calculated content of NSG (*a*), microvesicles (*b*) and vacuoles in the endings (*c*) of the neural lobe. The values are calculated from the volume proportion data assuming that the endings comprise 12.07% (ref. 13) of a total embedded neural lobe volume of 0.566 mm^3 (ref. 11). The Holmes correction has been applied to the neurosecretory granule data. Open columns, control experiments (5.6 mM KCl); hatched columns, stimulated preparations (56 mM KCl).

The amount of hormone released from one neurohypophysis during 15 min of incubation in the high-K^+ solution was 128 mU. Since each NSG contains 6×10^{-8} mU hormone[11], such a release would be represented by the content of 2.1×10^9 NSG. We calculate from the stereological data that the granule depletion from the endings alone represents a loss of 1.7×10^9 NSG, and redistribution of NSG within the neurosecretory process would undoubtedly make this a minimum figure for the total granule loss. There is thus surprisingly good agreement between the data derived by stereology and bioassay.

After release there is no detectable change in the volume

Table 1 Comparison of the proportional volume occupied by neurosecretory granules, microvesicles and vacuoles in neurosecretory endings of the neural lobe in control (5.6 mM K^+) and stimulated (56 mM K^+) preparations

		Control (5.6 mM K^+)	Stimulated (56 mM K^+)
Neurosecretory granules	ALL	18.8±0.9 (201)	11.9±0.9 (207)*
	BM+	17.6±1.1 (137)	8.7±0.8 (137)*
	BM−	20.7±1.8 (64)	20.3±2.1 (70)
Microvesicles	ALL	7.2±0.5 (201)	7.3±0.6 (207)
	BM+	6.8±0.6 (137)	8.2±0.8 (137)
	BM−	7.8±1.0 (64)	5.4±0.9 (70)
Vacuoles	ALL	1.2±0.4 (201)	3.6±0.5 (207)*
	BM+	1.6±0.6 (137)	4.2±0.7 (137)*
	BM−	0.2±0.1 (64)	2.2±0.5 (70)*

Values are percentages of nerve ending profile area occupied by the organelle (mean ± s.e.m.) The number of endings studied is given in parentheses. ALL, data for all nerve endings; BM+, data for those nerve ending profiles transecting the contact with the basement membrane; BM−, data for those ending profiles not transecting the contact with the basement membrane.

*Difference between control and stimulated, $P < 0.001$ by Student's *t* test.

occupied by microvesicles, but the volume of uptake by the newly formed vacuoles in the endings would be 1.5×10^6 µm^3 per neural lobe. This would represent about 41% of the volume of the NSGs released and is in fairly good agreement with the reported volume uptake (3.1×10^6 µm^3) determined by extracellular tracers[4].

The results therefore indicate that a major route for the recapture of membrane after exocytosis of NSG is by vacuoles of a similar size to NSG, but do not demonstrate any significant recapture by microvesicles. They thus contrast sharply with the findings of Nagasawa *et al.*[2] who report that, in the rat and hamster neurohypophysis, stimulation of hormone release results in uptake of horseradish peroxidase into microvesicles; they did not, however, quantify this effect. In the rat neurohypophysis, incubation in depolarising conditions results in an uptake of horseradish peroxidase and radioactive extracellular markers into the nerve endings[4]. Because this uptake was abolished when hormone release was blocked by Ca^{2+} antagonists, this is consistent with the concept of an exocytosis–endocytosis coupling mechanism.

In conclusion, our results highlight the fact that the model of membrane retrieval exclusively by way of microvesicles at neurosecretory axon terminals[2,12] cannot be generalised. Indeed our results show that after stimulation of hormone release there was no detectable change in the microvesicle population. Neurohypophysial vacuoles[3,4] can be a major route for recapture of membrane. The increase in size of the vacuoles after stimulation suggests that the size of the membrane-recapture organelle is a function of the intensity of secretion. Although larger vacuoles would be less efficient at storing membrane in a given volume, it would be expected that membrane retrieval into the larger organelles would be energetically a less costly process and would have the advantage of maintaining the volume of the endings relatively constant in the acute situation.

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Crystallisation of DNA-dependent RNA polymerase from *Thermus thermophilus* HB 8

BACTERIAL DNA-dependent RNA polymerase (nucleoside triphosphate–RNA nucleotidyltransferase; EC 2.7.7.6) consists of at least four different subunits (α, β, β', and σ) and the subunit composition of the holo-and core enzymes are $\alpha_2\beta\beta'\sigma$ and $\alpha_2\beta\beta'$, respectively. These subunits play different roles and the overall reaction catalysed by this enzyme is well organised and highly specific. To understand the mechanism of transcription, a precise knowledge of the structure of this enzyme is indispensable. The structure of RNA polymerase has been studied by small-angle X-ray analysis[1] and electron microscopy[2-6]. Data obtained using these methods, however, are not satisfactory, and even contradict each other. X-ray crystallography seems to be[7,8] the most powerful method of analysing the three-dimensional structure of proteins. Since RNA polymerase has not yet been crystallised, however, it has been impossible to apply this technique. We have recently isolated the core enzymes and holoenzymes of RNA polymerase from the thermophilic bacterium, *Thermus thermophilus* HB 8 (ref. 9). Both enzymes are quite stable not only against heating but also against denaturation by various detergents. One of the reasons why crystallisation of RNA polymerase has not yet been achieved in spite of many publications concerning extensive purification of the enzyme from various sources, is probably the instability of the enzyme. As a first step towards X-ray crystallographic analysis of RNA polymerase, we tried to crystallise the thermophilic core enzymes and holoenzymes in the hope that their extreme stability might help the crystallisation of the enzyme. We have succeeded in crystallising the core enzyme but not the holoenzyme.

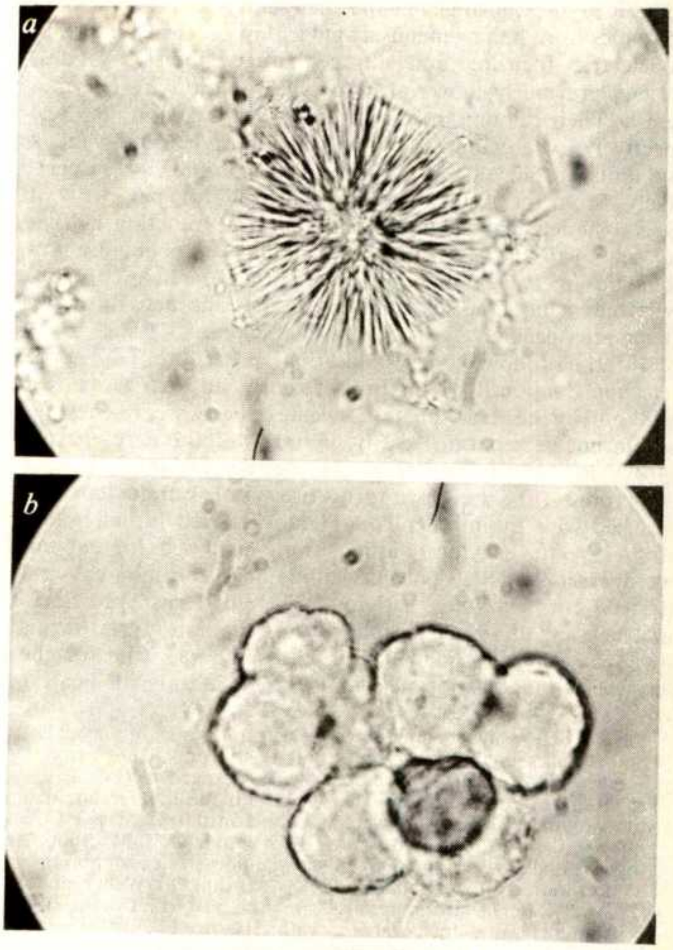


Fig. 1 Photomicrograph of crystals of DNA-dependent RNA polymerase (core enzyme) from *T. thermophilus* HB 8: *a*, needle crystals; *b*, spherulites (about × 400). For crystallisation, the core enzyme (5 mg ml^{-1}) was dialysed in a small dialysis bag against 0.01 M Tris–HCl, 0.01 M $MgCl_2$, 0.1 mM EDTA, 2-mercaptoethanol, containing 25% saturated (at 25 °C) ammonium sulphate, *p*H 8. The dialysis bag was placed in a test tube together with 15 ml of the dialysate. An appropriate amount of the saturated ammonium sulphate solution, made by further addition of solid ammonium sulphate to the dialysate, was added without stirring the tube, so that the final concentration of ammonium sulphate after equilibrium becomes 55% or more. The test tube was covered with polyethylene film and left at 25 °C. Crystallisation was completed in a few weeks.

The thermophilic RNA polymerases, holo- and core enzymes, were isolated from *T. thermophilus* HB 8 as described previously[9]. The purified holoenzymes and core enzymes were homogeneous as judged by gel electrophoresis, isoelectric focusing and ultracentrifuge studies. As described previously, however, two types of the core enzymes exist. Their subunit composition is $\alpha_2\beta\beta'$ and $\alpha_2\mathrm{x}\beta'$, respectively, where the molecular weights of α, β, β', and x subunits are 42,000, 140,000, 180,000 and 100,000, respectively. From the results of our reconstitution experiments, it was assumed that subunit x has the same function as that of subunit β and is derived from subunit β probably by proteolytic digestion (our unpublished results). These two core enzymes cannot be distinguished by specific activity or by template specificity.

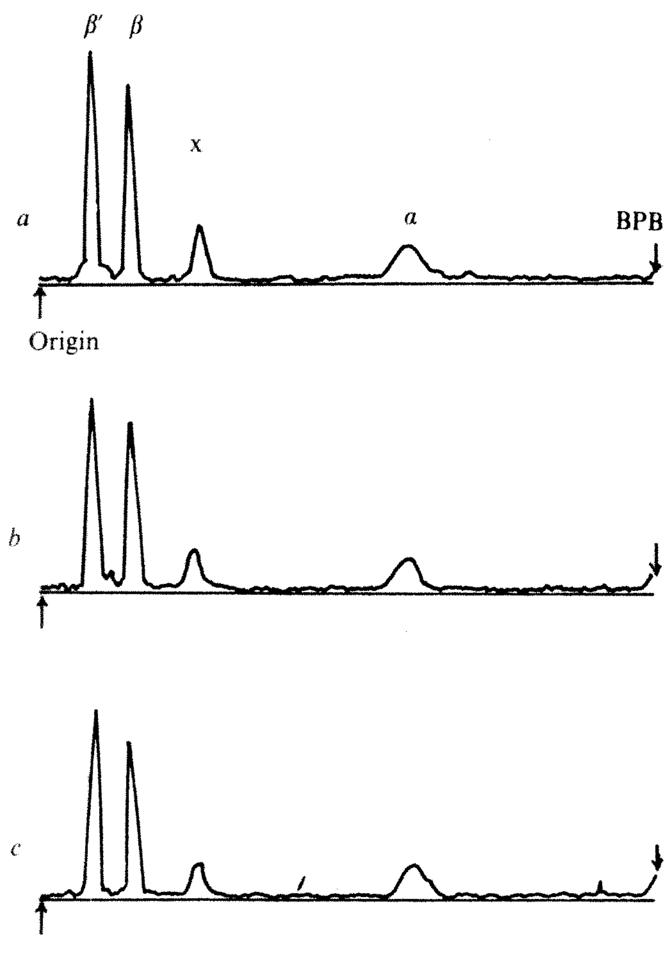
Table 1 Specific activity of the core enzyme of RNA polymerase from *T. thermophilus* HB 8, and its crystals

	Specific activity ($\times 10^{-4}$ U mg^{-1})
Core enzyme of RNA polymerase	2.12
Needle crystals	1.71
Spherulites	1.55

The two forms of crystal were dissolved in buffer as described in Fig. 1, and these solutions were assayed with poly d(A-T) as template. The assay mixture (0.10 ml) contained 0.05 M glycine-NaOH, *p*H 8.2, at 65 °C, 10 mM $MgCl_2$, 2 mM $MnCl_2$, 0.1 mM EDTA, 0.1 M KCl, 0.01 M 2-mercaptoethanol, 0.4 mM each of ATP, GTP and CTP, 0.4 mM ^{3}H-UTP (1–2 mCi mmol^{-1}) and 4 μg poly d(A-T) and 1 μg enzyme. After incubation for 10 min at 65 °C the reaction mixture was chilled in ice-cold 10% TCA. The precipitates were collected on a Millipore filter, and washed with ice-cold 5% TCA. The filters were immersed in a toluene based scintillation fluid and the radioactivity was counted with a Packard Tri-Carb 2425 liquid scintillation spectrometer. One unit of RNA polymerase activity is defined as the amount of protein which catalyses the incorporation of 1 nmol UMP into RNA in 60 min at 65 °C in the conditions described above. The amount of protein was calculated from A_{280} and A_{260} according to Kalckar[11].

In the following crystallisation procedure, the core enzyme fraction (3 : 1 mixture of $\alpha_2\beta\beta'$ and $\alpha_2\mathrm{x}\beta'$) was used without further fractionation, because the two core enzymes could not be separated easily[9]. Crystals of the core enzyme grow in two forms (Fig. 1)—needle crystals (*a*) and spherulites (*b*). These two forms of crystals were extensively washed with the mother liquor and dissolved in buffer (see Fig. 2). The enzyme solutions were analysed for subunit composition by SDS polyacrylamide gel electrophoresis and tested for enzyme activity. The patterns of SDS–polyacrylamide gel electrophoresis of the two crystal forms (Fig. 2) were almost identical. The same was true for the core enzyme fraction used for crystallisation, indicating that both core enzymes crystallised in two forms. Since the amount of x in both crystal forms seems, however, to be slightly lower than that in the original fraction, the $\alpha_2\beta\beta'$ type core enzyme may crystallise more easily than the $\alpha_2\mathrm{x}\beta'$ type core enzyme. Specific activities of crystals (Table 1) were slightly lower than that of the original core enzyme, but it can be concluded that these crystals have the core enzyme activity.

Fig. 2 SDS–polyacrylamide gel electrophoresis of crystals and the original core enzyme of RNA polymerase from *T. thermophilus* HB 8: *a*, core enzyme; *b*, needle crystals; *c*, spherulites. After washing extensively with the mother liquor, the two forms of crystal were dissolved in 0.01 M Tris-HCl, *p*H 8.0, 0.01 M $MgCl_2$, 0.1 mM EDTA, and 0.1 mM dithiothreitol, and analysed by SDS–polyacrylamide gel electrophoresis (concentration of acrylamide 7.5%) according to Weber and Osborn[10]. After electrophoresis, gels were stained with Coomassie brilliant blue, and traced at A_{620} with a Joyce Loebl Chromo Scan FS 200. BPB indicate the position of the bromophenol blue marker dye.



These results indicate that both are crystals of the core enzyme of DNA-dependent RNA polymerase from *T. thermophilus* HB 8, and have essentially identical compositions and activities. Protein crystals which grow as hedgehog-like aggregates of needle crystals (Fig 1*a*) frequently develop into spherulites (Fig. 1*b*).

To obtain core enzyme crystals suitable for X-ray analyses and to crystallise the holoenzyme precipitated in the above conditions, further improvement of the conditions for crystallisation is necessary.

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Nature Index and Binders

The **Index** for 1975 is now available, price £2.25. Copies of the 1974 index are still on sale, price £3.00. **Binders** for the journal are also available at £7.00 for four (a year of *Nature* fits into four binders).
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reviews

Forests and their genetics

Tropical Rain Forests of the Far East. By T. C. Whitmore. With a chapter on soils by C. P. Burnham. Pp. xiii+282. (Clarendon: Oxford; Oxford University: London, December 1975.) £12.50.

THIS is a superb book I dearly wish that it had been available before I made my first visit to a tropical rain forest. The title is accurate but unduly modest. The book indeed treats tropical rain forests of the Far East exhaustively, but in a surprisingly compact and literate form. The author's approach, however, is so broadly interpretive that much of what he says gives equal insight into other tropical forests.

In addition to chapters on geography, climate, and forest structure, there are excellent separate chapters and dispersed notes on silviculture, animals, and the interactions between plants and animals.

Much of the interpretive study of tropical forests has suffered from overgeneralisation followed by nihilistic reaction. Whitmore gives refreshingly balanced discussions of topics such as storied vegetation, lateritic soils, buttressed roots, and compound leaves. He reviews recent theories of the structure and economy of tropical forests with a healthy mixture of enthusiasm and agnosticism. His extensive field experience is apparent in many practical hints about what one should look for when identifying trees or reading a landscape.

The book is abundantly illustrated with elegant photographs, each taken under technically taxing conditions and each showing its ecological point remarkably well Cross-references among chapters are unusually apt and easy to follow. The bibliography is copious, selected with acute judgement, and up to date within a year of its publication. The free use of botanical cryptology is a deterrent to the wide 'browsership' that the book deserves; but it is not an insurmountable difficulty, since arcane terms are defined where they first occur A glossary would have helped.

Whitmore ends most of his critical discussions with an explicit list of data that are needed to evaluate interpretations of previous observations His book should therefore be especially valuable to graduate students and advanced undergraduates. It is both successor and complement to P. W. Richard's classic *The Tropical Rain Forest* (1952, Cambridge University Press)

A final section on human use is a succinct, rational, and eloquent plea for conservation of some remaining unexploited forests, and for applying natural history and common sense to the management of exploited areas.

Henry S. Horn

Introduction to Forest Genetics. By Jonathan W. Wright. Pp. xvi+463. (Academic: New York and London, February 1976.) $19.50; £10.15.

STUDY or use of genetic variation in forest trees began early in the last century but systematic application of genetic principles to the domestication of trees began only 50 years ago. Through the last 30 years there has been a dramatic increase in activity with programmes for population testing and selective breeding being initiated in many countries, with many species, and for many different types of forest products.

Professor Wright has long been one of the leading practitioners and teachers of forest genetics and tree improvement in North America where, together with northern Europe, most of the early work was done and where the larger and most advanced programmes are located. One of the few criticisms of this book arises from that; the bulk of the examples and the references quoted are from the temperate regions of North America and Europe with some from Japan, New Zealand and South America; tropical species and areas in which there is now much activity and rapid progress, receive scant attention. Although this may detract from the value of the book as a worldwide review it does not affect the principle aim, which is to provide an introductory text for future professional tree breeders.

In 1962, at the request of the United Nations Food and Agriculture Organisation Professor Wright prepared *Genetics of Forest Tree Improvement* (FAO Forestry and Forest Product Studies No 16, Rome). Until now this has remained the only textbook in English on the subject and for several years it has been unobtainable.

The present book is a revision of the 1962 study with updated examples and illustrations and none of the errors that marred the first book. It does not assume great previous knowledge of genetics or statistics; throughout, the book has a pleasing balance between theory and practice. Less pleasing is the imbalance in the bibliographic citations in the various chapters; some controversial statements are made dogmatically and unsupported by literature citations, whereas others are supported unnecessarily by large numbers of references.

The subject matter covers basic concepts of Mendelian and quantitative genetics in the first five chapters, although a consideration of chromosomes themselves (polyploidy, aneuploidy and haploidy) is deferred until the last (nineteenth) chapter. The remaining chapters are the core of practical tree improvement covering the four most common approaches to tree improvement: individual tree selection and breeding; provenance testing; species and racial hybridisation; introduction of exotics. A useful glossary and adequate index are provided.

This book holds something of value to all foresters and crop breeders. It is a basic reference for tree breeders and essential for all courses in forest genetics and tree improvement.

J. Burley

Nuclear magnetic resonance

NMR in Biological Research: Peptides and Proteins. By Kurt Wüthrich. Pp. xii+379. (North-Holland: Amsterdam and Oxford; American Elsevier: New York, 1976) $45.95; Dfl.115.

THE increasing interest in the application of nuclear magnetic resonance (NMR) techniques to biological problems is reflected in the spate of books and review articles which have recently appeared on the subject. Professor Wuthrich's book is a valuable addition to this collection in that he has produced an ideal text for introducing the chemist or biochemist into this area of research. The main emphasis of the book is on illustrating the different kinds of biologically relevant information which can be obtained from NMR methods By restricting himself to studies of peptides and proteins he has been able to present a comprehensive and coherent account of the potential of the technique in these systems. Almost all the principles of NMR biological applications are contained in such studies so the student should have little difficulty extending them to other systems.

The book deals with the theoretical aspects of NMR in an elementary phenomenological fashion and provides the key references required to provide more detailed knowledge in these areas. The text covers studies of structure, conformation and molecular dynamics of peptides and proteins, ^{13}C, ^{15}N and other nuclei studies related to these systems, and also considers the effects of paramagnetic centres on proteins Although no attempt is made to present a comprehensive coverage of the literature, several well-chosen examples are discussed in detail for each of the applications considered. Many of these examples are from recent work in Professor Wuthrich's laboratory In particular, his excellent ^{1}H spectra of basic pancreatic trypsin inhibitor have been used to good effect to illustrate a wide range of applications in protein NMR studies.

The book contains a good deal of useful NMR reference data for amino acids, peptides and proteins The author has paid special attention to ^{13}C (50p) and ^{15}N (23p) biological applications — areas inadequately covered in other texts. I recommend this book to anyone interested in assessing whether NMR can help with their biological problems. **J. Feeney**

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Blood vessels in perspective

Blood Vessels. (Biological Structure and Function.) By W. J Cliff. Pp ix+214 (Cambridge University Cambridge and London, April 1976.) £10

THE object of this monograph is to show how the different anatomies of the different types of blood vessel are related to their several functions In addition, an attempt is made to deal with the alterations in wall structure and function that result from pathological processes It is partially successful

The author works in the Department of Experimental Pathology at the Australian National University, and his background enables him to look at blood vessels in pretty broad perspective. The result is a useful mix of structure and function, although structure gets the stronger emphasis Certainly a broad view is necessary when attempting to review in depth the anatomy, ultrastructure, physiology, pharmacology and pathology of all types of blood vessels, the author admits that no one person could carry out the task to perfection. Thus, each specialist will feel some dissatisfaction when he reads the parts which deal with his own field—a wrong emphasis here, an important point missed there, and sometimes a superficial treatment which betrays misunderstanding. These weaknesses are more apparent when the author discusses function.

The main chapters deal with the cellular, extracellular and ancillary structures connected with the walls of blood vessels, but there are also chapters dealing with the vessels as functional units Extensive use of comparative studies is made to indicate the way vascular structure and function have been developed The book contains 67 figures, the majority of which are halftone photographs of histological sections and electron micrographs. There are also 34 pages of references, the most recent being from 1972 journals

For a refreshing and overall view of blood vessels, the book has much to commend it The author has a pleasing style of writing, which lends itself to easy reading. The cover states that the book is aimed primarily at anatomists, physiologists and pathologists they will learn and derive enjoyment from reading it. **I. C. Roddie**

Water facets

Water: A Comprehensive Treatise. Edited by Felix Franks. Vol. 4: Aqueous Solutions of Amphiphiles and Macromolecules. Pp xxi+839. Vol. 5: Water in Disperse Systems. Pp. xvi+366. (Plenum: New York and London, 1975) $44.50 each volume.

THESE volumes bring to a close the monumental compendium on water which Felix Franks has brought into being with the aid of two dozen or so associates. With this treatise in hand, the reader can certainly get an initial grip on the literature of almost any facet of this multidimensional field, although many of the chapters leave one less than satisfied with the current level of understanding of the problem. This is, however, more a reflection of the overwhelming complexities of the potential interactions of water with the materials of the inorganic, organic and biological worlds, than on the authors of this work.

Volume 4 and 5 differ from their predecessors in point of view; here water is no longer the subject, but rather the all encompassing 'environment'. Students of biological macromolecules have considered it natural to examine them in water as the native habitat, and have only lately come to realise that many of the properties of these macromolecules actually arise in large part as a *consequence* of their existence in the aqueous milieu. On the other hand, the field of synthetic polymer chemistry has developed primarily in organic solvents, and thus in this area the role of water in changing polymer properties and conformations is both explicitly better discriminated, and less well explored.

Volume 4 opens with a chapter by Felix Franks on Hydrophobic Interaction, summarising the theoretical and experimental approaches to small molecules in water which have been taken in an attempt to understand hydrophobic bonding as it might stabilise macromolecular conformations. This serves as a useful complement to the long chapter by Eagland on Nucleic Acids, Peptides, and Proteins, in which is assembled an enormous volume of information on these macromolecules as they exist in aqueous solutions. The merging of these approaches is largely left to the reader in this volume, although it might be worth pointing out that these authors have recently combined forces to bring about an excellent synthesis in an article in *Critical Reviews of Biochemistry* (**3**, 165, 1975).

Volume 4 also contains thorough summaries of the present status of our understanding of Lipids by Hauser, and of Polysaccharides by Suggett. The remainder of this volume comprises chapters on synthetic systems. That on Surfactants by Kresheck provides a nice summary of micelles and micelle-forming molecules; the chapter on Dyestuffs by Duff and Giles emphasises the spectroscopic effects of dye aggregation and stacking interactions, and as such provides a useful and less frequently presented starting point for considerations of the interactions involved in 'stacking' nucleic acids, etc. The final chapter on Synthetic Polymers, by Molyneaux, helps us to organise this vast literature.

In some senses volume 5, which deals largely with what seem to be totally intractable systems, represents the more unusual of these volumes. It opens with a detailed but well presented chapter on Colloidal Systems, again by Eaglund, which pulls together many aspects of the present state of colloid chemistry. The chapter by Clifford on Water in Capillaries and Thin Films, is, as one might expect, mostly descriptive. It presents, however, one of the most balanced and coherent summaries I have read of two very controversial areas: the status of anomalous water ('polywater'), and of water in cells and tissues. Forslind and Jacobsson use the chapter on Clay–Water Systems in part to provide us a summary of their point-of-view on water structure and water–surface interactions. The remainder of the volume is devoted to chapters on water in very special environments: including Foams and Emulsions (Phillips), Solid Surfaces (Zettlemoyer, Micale and Klier) and Biopolymer Surfaces (Berendsen). Here, as one might expect, the behaviour of water is primarily modulated by the molecular properties of the material comprising the surface or interface at issue, and less by the properties of bulk water.

Overall, Franks and his colleagues have done well by us, providing an appreciation of the ubiquitous effects of water in controlling almost all of life processes, and at the same time providing us with a point at which to approach the various problems of concern to individual investigators. This treatise should be available to all serious research workers concerned with chemical or biological problems in a primarily aqueous world.

Peter H. von Hippel

Erratum. In the review of *Lunar Science: A Post-Apollo View* (S. R. Taylor) by S. K Runcorn (*Nature*, **260**, 813, 1976), paragraph 3, line 20 should read " . . . implausible—Velikovsky's predictions that . . .".

Crystallinity of macromolecules

Macromolecular Physics. Vol. 2. Crystal Nucleation, Growth, Annealing. By Bernhard Wunderlich. Pp. xi+461. Academic: New York and London, March 1976.) $46.50; £25.60.

THIS is the second volume in a series so far devoted to phenomena associated with the crystallinity of macromolecules. Volume 1 having dealt with structural aspects, the present volume covers the crystallisation process itself in three consecutive chapters headed by the three subtitles of the book The book has been long awaited in response to a very real need. This is a work primarily for the active researcher in the field but should also be most useful as a reference book for wider readership in search of information. The book follows no traditional pattern as regards the detailed subject matter or its organisation, and as such is without rival. Its function, as I see it, is intended to be twofold: compilation of knowledge as it exists at present; placing this knowledge in the framework of fundamentals.

As regards my first point, the book is of inestimable value for anybody involved in the subject. Much of the information contained is outside the scope of the usual 'handbook' classification and simply cannot be obtained elsewhere except for the widely scattered original papers. As in volume 1 the Tables are particularly of value in this respect and so is the comprehensive bibliography giving full titles. Also for a book of this size the information content is remarkably up to date

In addition, the unconventional juxtaposition of an unusually wide range of different subjects in support of particular themes is both informative and stimulating. For example, it contains material on liquid crystals, on macroscopic polymer crystals obtained from polymerisation in the solid state, on simultaneous polymerisation and crystallisation a particularly unique feature of the book), on organic materials, and even on elements forming polymers—all subjects which although relevant, normally do not feature in the standard polymer textbooks.

To achieve the second purpose the author goes back to the fundamentals of phase transitions before his treatment of polymers. Whether these extensive introductions would stand their ground as independent treatises may well be questioned. Even so, they represent a laudable endeavour for a book on polymers. It is a further question how long the present theoretical framework will remain accurate, or even pertinent, as the subject is still in a state of development; there have been changes even since the book went to the press. Even so, the author is to be praised for his courage in investing all this work in a subject still in a state of flux

Although, as in volume 1, there is still too much uncritical juxtaposition of facts, there are now certain firm anchor points: altogether a more deliberate attempt has been made to provide critical guidance when presenting conflicting information than in the first volume.

The book is beautifully and carefully produced. As in volume 1, the author has even gone to the trouble of redrawing diagrams to suit his points and to achieve the high aesthetic standards he set for himself.

Altogether it is a welcome and laudable effort.

A. Keller

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Insect ecology

Insect Ecology. By Peter W. Price. Pp. xii+514. (Wiley-Interscience: New York and London, October 1975.) £8.10.

THIS book is valuable as the only recent, complete text of insect ecology. Other current works stress either insect population dynamics or applied aspects of the subject. A cursory glance is encouraging; the author has read very widely, selected a balanced bibliography (from both sides of the Atlantic) and covered all the major topics one would hope to see, with the possible exception of insect movements (population structure, migration, and so on).

A closer look reveals a high vertebrate content, and initiates a suspicion that this may be not so much an insect ecology text as an ecological compendium, with examples to illustrate each concept selected from insects where possible. This suspicion materialises as being partly unfounded, in that the extensive coverage of the behaviour of vertebrate predators helps us to understand the nature of selection acting on insects in their capacity as prey. In some other chapters, however, the vertebrate content is unnecessary and could be misleading, since, in cases in which an ecological generalisation can only be illustrated with vertebrate examples, there may be good reason for this. The generalisation may not extend to insects, especially to those with complex life cycles. Some critical evaluation of the extent to which this is true would have been valuable in an insect ecology text.

Some of the chapters have problems which I hope will be corrected in future editions. Here are three examples. The "Reproductive Strategies" chapter persistently implies that fecundity may evolve (in either direction) to balance imposed changes in mortality, a hypothesis which is wrong, unless accounted for in terms of group selection, which it is not.

The "population dynamics" chapter seems to use the words "limit", "control", "regulate" and "determine" interchangeably and without definition. This is a pity, considering the semantic difficulties which have arisen in the past in this field in relation to the use of these words. It results in a very unclear presentation of the 20-yr-old "density-dependence" debate, since one of the causes of dispute stemmed from the fact that members of opposing camps were using the word "control" in different senses. It also results in repeated use of the phrase "density-independent regulation", which, in current usage, is a contradiction in terms.

The chapter on social systems and behaviour seems unnecessarily abstract, since it does not *describe* the social organisation of any insect. The theoretical content is not well balanced, and sometimes becomes self-contradictory through accepting incompatible conclusions of different authors. For example, the basic hypothesis of group selection is accepted in the second paragraph on p. 319 and rejected at the bottom of the same page.

In sum, this is a very useful book, an excellent source of references, but one in which the theoretical arguments must occasionally be taken with a pinch of salt.

Michael C. Singer

The Filamentous Fungi

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Effects of cold on man

Man in the Cold. (A Monograph in the Bannerstone Division of American Lectures in Environmental Studies.) By Jacques LeBlanc. Pp. x+195. (Thomas: Springfield, Illinois, November 1975.) $15 50.

PROFESSOR LEBLANC is well-known for his studies on the effects of cold on man, so a book by him on the subject must be of interest to all those concerned with environmental factors. Although on the dust sheet it is proclaimed that this book covers the whole field, and is a comprehensive review of relevant work, it may be doubted whether Professor LeBlanc would make such a claim. The book is quite short, less than 20,000 words, but is lavishly illustrated with graphs, and so on (There are so many it is not surprising some of the figure legends have been interchanged.)

In the relatively brief text, Professor LeBlanc sensibly devotes a good deal of space to his own work, and this is certainly the most interesting and useful part of the book. He describes the experiments he and his colleagues have carried out on the effects produced by immersion of the face in cold water and comparing these with the changes observed when the hand is put in cold water at about 4 °C. Lewis showed in 1930 that the blood flow in the fingers shut off virtually completely within seconds after immersion in ice water. The fingers became painful and the pain increased for several minutes; then, after 5–6 min, the pain stopped and it felt as if the water temperature must have been raised. This change was due to a sudden vasodilatation and a large blood flow.

Cold-induced vasodilatation is a remarkable phenomenon, and, as Professor LeBlanc has shown, in people who habitually expose their hands to cold water, the vasodilatation comes on more rapidly and is larger than in unaccustomed subjects. Heart rate and blood pressure increase as pain increases, and return to control values during vasodilatation In contrast, when the face is immersed in water at 4 °C there is pain and an increase in blood pressure, but heart rate slows. This effect is not observed if the face is immersed in warm water at, say, 30 °C. There is no change in heart rate or blood pressure, and there is no pain. The bradycardia in the cold can be evoked by cold air as well as cold water. Professor LeBlanc suggests that the pain and distress often experienced by patients recovering from an attack of coronary heart disease when they walk out of doors on a cold day facing the wind could be due to this bradycardia combined with an increase in blood pressure.

It is undoubtedly useful to have a detailed account of the work of Professor LeBlanc, and for this reason the book is recommended. It is not a complete review of what is by now a very large body of research work dealing with the effects of cold on man

O. G. Edholm

nature

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